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Editor
ROY WALDO MINER

NUTRITIONAL FACTORS AND LIVER DISEASES

BY

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NUTRITIONAL FACTORS AND LIVER DISEASES*

Cons l ng Ed r a l Co fere e C a rm a KLAUS S ARZ

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Part II Liver Necrosis

INTRODUCTION: LIVER NECROSIS VERSUS FATTY LIVER AND CIRRHOSIS

By Klaus Schwarz

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> Part of our knowledge we obtain direct and part by argument.
> —JOHN MAYNARD KEYNES, *A Treatise on Probability*

It is, of course, a great pleasure to write the introduction to this monograph based on the Conference on "Nutritional Factors and Liver Diseases," which it has been my privilege to plan and to organize. The concepts and intentions which have guided this endeavor can be outlined as follows. Within the limitations of a two-day meeting and the following collection of papers presented, an attempt has been made to assemble a very broad spectrum of our present knowledge and of all current trends in research on nutritional liver disease, to put before you a real "symposium." What appears more important, however, is that an effort has been made to put our various pieces of knowledge together in such a way as to form a clearer picture out of the rather chaotic development which this field has undergone. This may permit us to visualize and to understand possible relations between heretofore isolated areas, to clarify, at least in part, some of the complex aspects of this field, which seemingly have been lacking integration so far.

To achieve this purpose, several heterogeneous fields had to be brought together and connected in a coordinated fashion, the two most important ones being biochemical research in experimental animals on one side and clinical and pathological research on humans suffering from dietary liver disease on the other. Workers not only from various scientific fields but also from different continents had to be brought together since the investigation of experimental dietary liver diseases has advanced most in North America and in Europe, whereas work in human nutritional liver injury has by necessity been carried out more intensely in Latin America, Asia, and especially in Africa where Cecily Williams made her classical observations on kwashiorkor in 1933.[1] Our appreciation is extended to all the workers from abroad who have contributed so effectively to the meeting and to this volume.

The guiding consideration in the arrangement of the following material is based on the results of animal experimentation. It is quite certain that, in experimental animals two completely different deficiency syndromes can be distinguished clearly. These are fatty liver and cirrhosis on the one hand, and acute (massive) liver necrosis preferably called necrotic liver degeneration,* on the other. These two disease entities can be produced separately and even occupy a mutually antagonistic position in response to various dietary factors which are protective (see below). According to this clear-cut separation, the conference program and this monograph are separated into two main

* "Degeneration" is defined as progressive deterioration of tissue in which its vitality is diminished, whereas "necrosis" means death of a circumscribed portion of tissue. Dietary necrotic liver degeneration is applied to define a deficiency disease which in previous publications has been called "hemorrhage," "hemorrhage and necrosis," or "liver degeneration." The defect is characterized by a degenerative change which eventually leads to acute extensive liver necrosis and death.

parts, one being devoted to fatty liver and cirrhotic diseases and the other to necrotic liver injuries Each main part, in turn, was scheduled originally to present first the results of animal experimentation and, secondly, the clinical investigations of corresponding nutritional liver injuries in the human A certain imbalance is evident, however, since experience with human fatty liver and cirrhosis due to malnutrition is abundant indeed, whereas knowledge of human necrotic liver disease due to malnutrition is quite inadequate as yet

Nutritional Liver Disease in Humans (Kwashiorkor)

It has become quite clear that nutritional disease of the liver occurs very frequently in small children and also in adults, especially in tropical and in subtropical areas, as part of the so called kwashiorkor syndrome The disease constitutes a world wide problem It is one of the most important dietary deficiencies in mankind today This is not yet sufficiently realized in some countries, partly due to indifference and partly due to lack of information and of communication It is often fatal and poses difficult and challenging problems in the field of clinical medicine and in nutrition research The disease is not really new It has been described under a great variety of names for example as kwashiorkor[2] culebrilla syndrome policarential, malignant malnutrition, distrophie de farineuse, and as far back as 1906 as Mehlnahrschaden[3] The African term kwashiorkor has been officially accepted by the Joint Expert Committee on Nutrition of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations

In the liver, the major features of kwashiorkor are fatty infiltration and cirrhosis These changes are apparently intimately related, if not identical, to fatty infiltration and cirrhosis in areas with temperate climates, particularly among alcoholics For this reason, the nutritional theories of the etiology of alcoholism, as well as the dietary aspects of the origin and the treatment of alcoholic fatty liver and cirrhosis have been considered as pertaining to the general theme of this volume The dietary factors which protect against human fatty liver and cirrhosis have not been elucidated as yet

Experimental Nutritional Liver Disease

In experimental animals, dietary liver diseases (i e, liver injuries produced [illegible]

describing "hemorrhages throughout the liver and "clear signs of icterus" which were produced by a cystine deficient diet was not realized until much later When du Vigneaud and his associates produced liver necrosis in rats on amino acid diets in 1939 they devoted only one paragraph in small print to the description of the phenomenon[7] Between 1939 and 1946 difficulties were encountered in the differentiation of these two types of liver diseases, and the resolution of these difficulties was seriously delayed by the international scientific blackout during World War II

In doing experimental research with purified diets, we have, allegedly, the

advantage of being able to deal with "pure" forms, with just one variable at a time, whereas the clinician deals with more complex situations. However, it is characteristic for both experimental fatty liver/cirrhosis and liver necrosis, that they are the result of rather complicated dietary deficiency situations. Both of these injuries are not the result of single dietary defects, but are caused by the simultaneous lack of several essential nutrients. In both diseases some of the major dietary factors have been identified, but hitherto unknown protective factors are also involved. Hence, it is understandable that research workers have not always succeeded in producing pure forms of these diseases. In 1939, papers appeared describing the occurrence of both liver necrosis and fatty infiltration/cirrhosis in the same animals. These had been kept for extended periods on low protein rations which were deficient in quite a variety of essential dietary components. Thus both types of nutritional liver disease were produced simultaneously, leading some workers to think that those lesions constituted a single deficiency syndrome. This somewhat handicapped progress in the clarification of the factors involved. In 1942 it was clear, established by Daft, Sebrell, and Lillie[8] that the two diseases were separate entities. This, however, did not prevent the subsequent publication of occasional papers which failed to discriminate between the fatty liver/cirrhosis syndrome and dietary necrotic liver degeneration. In Europe, during this same period, several dietary methods were found which produced necrotic liver degeneration at high incidence and in a reproducible manner.[9, 10, 11] Signs of fatty infiltration/cirrhosis were not seen since the rations were supplemented with choline. The necrotic deficiency disease was found to be prevented by cystine[9] and in 1944 vitamin E was disclosed to be highly protective.[12] More recently a third, not yet identified factor, Factor 3, has been shown to be involved.[13] Each one of these three substances alone affords protection

TABLE 1

Disease process	LIVER NECROSIS	FATTY LIVER CIRRHOSIS
	degenerative metabolic change of the liver parenchyma leading to sudden, acute attack of massive necrosis	fatty metamorphosis of liver with slowly developing fibrosis cirrhosis
Residual signs	No fatty infiltration No fibrosis postnecrotic scarring	No (massive) necrosis cirrhosis
Protective factors		
Cystine*	protects	*enhances*
Vitamin E	protects	without effect
Factor 3	protects	not known
Choline	*enhances*	protects
Betaine	*enhances*	protects
Methyl group precursors	*enhances*	protects
Vitamin B_{12}	*enhances*	protects
Folic acid citrovorum factor	*enhances*	protects

* Methionine has been shown to be protective against fatty liver and cirrhosis; it contains a labile methyl group. It is also slightly protective in dietary necrotic liver degeneration since it is partly transformed into cystine in intermediary metabolism.

Thus it became obvious that the group of dietary factors which prevents necrotic liver degeneration is completely different from the group of factors which protects against fatty liver/cirrhosis Furthermore it could be shown that substances which prevent the development of fatty liver and cirrhosis such as choline, betaine, precursors of one carbon units, vitamin B_{12} and folic acid/citrovorum factor enhance the development of the necrotic liver injury [14] On the other hand, cystine, which protects against necrotic degeneration, accentuates the fatty liver/cirrhosis syndrome The situation is summarized in TABLE 1

Regarding the Relationship of Experimental and Human Nutritional Liver Disease

It is evident from this monograph that the various phases of experimental and of human nutritional liver disease can be put together in such a way that a great number of parallels become visible This is particularly true for the fatty liver/cirrhosis diseases However, there are also obvious discrepancies As to necrotic liver injury, the situation is much less clear, the occurrence of nutritionally induced necrotic disorders in the human remains to be established I want to state explicitly that the obvious parallelisms do not necessarily [illegible]

clarified Knowledge of human nutritional disorders of the liver, by comparison, is much less advanced The more or less close parallelisms between human liver disease and the two main types of experimental dietary liver injury have of course led to conjecture and to premature discussion of the question of how much human liver disease is induced nutritionally and what might be done with liver protecting factors in therapy As long as we do not know all the dietary factors necessary to protect, these questions cannot be answered Early hopes, for example, that choline, which is protective against fatty liver and cirrhosis in the rat, would prevent or cure fatty liver and cirrhosis in the

Between different species or even between different strains of the same kind, differences in the relative importance of these various essentials are naturally bound to occur, and we should not expect the human to respond in all details exactly as the rat does

The conclusion which can be drawn from this example is that much more careful studies are necessary before the true relationship between experimental and human dietary liver injury can be ascertained. Such relationship is bound to exist, but at present we can not recognize it precisely It is hoped that a full exchange of knowledge and of opinions—often controversial—will be helpful to both the laboratory investigator and the clinician This exchange is what has been attempted here

Among biochemists pathologists, and doctors there are differences of approach and of thinking with resulting possibilities of misunderstanding There are also pitfalls in terminology and in semantics Bringing these groups together constituted something of an experiment in itself, one which appeared well worth trying

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Much has been achieved in experimental nutritional liver disease even though ere are still many important questions and problems which have not been arified Knowledge of human nutritional disorders of the liver, by com rison, is much less advanced The more or less close parallelisms between iman liver disease and the two main types of experimental dietary liver injury ve of course led to conjecture and to premature discussion of the question how much human liver disease is induced nutritionally and what might be ne with liver protecting factors in therapy As long as we do not know the dietary factors necessary to protect, these questions cannot be answered irly hopes, for example, that choline, which is protective against fatty liver d cirrhosis in the rat, would prevent or cure fatty liver and cirrhosis in the man have not been fulfilled. This has led some to discredit the nutritional e of approach Since then we have learned that numerous factors other

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Part I. Fatty Liver and Cirrhosis

EXPERIMENTAL DIFFERENTIATION BETWEEN LIVER NECROSIS AND LIVER CIRRHOSIS AND SOME DIETARY FACTORS AFFECTING THEIR DEVELOPMENT

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It is now generally recognized that dietary liver cirrhosis and dietary liver necrosis are separate and distinct entities, both etiologically and morphologically. That is not to say that death of cells does not occur as part of the cirrhotic process or that fibrosis may not follow necrosis. Most observers of experimental dietary cirrhosis have reported the presence of scattered necrotic liver cells. I believe that none would contend, however, that massive necrosis is part of this process. Extensive scarring following massive necrosis has been observed also in several laboratories. We at the National Institutes of Health, however, have never observed diffuse fibrosis following dietary necrosis and the same appears to be true of most other groups of investigators. Doctor Hoffbauer will discuss this matter at some length in a later paper. It suffices to say here that there are two distinct processes, experimental dietary cirrhosis and experimental dietary necrosis, which may occur either separately or in the same liver in rats which have received certain deficient diets.

At this time I should like to review briefly the historical background of this concept and present some additional data in its support. In addition, I should like to discuss a few experiments carried out in our laboratory on the influence of dietary ingredients other than those previously implicated, on the development particularly, of fatty livers and etiologically related lesions.

The first clear differentiation between dietary liver cirrhosis and dietary liver necrosis was made in 1942 by Daft, Sebrell, and Lillie.[1,2] In our first publication on this subject, in *Federation Proceedings*,[1] we stated, "Under the conditions of our experiments choline prevents the cirrhosis, cystine prevents the hemorrhagic necrosis, and methionine prevents both the cirrhosis and the hemorrhagic necrosis. It appears, therefore, that the cirrhosis and the hemorrhagic necrosis are separate and distinct pathological entities." In our more complete report published a few months later in the *Proceedings of the Society for Experimental Biology and Medicine*[2] our conclusions were worded somewhat more conservatively, but the reasons for our belief that we were dealing with two distinct deficiency syndromes were clearly stated. The evidence on which our conclusions were based consisted of morphological observations and the results of dietary experiments. In regard to the morphological findings, we stated "Some rats on this same diet developed hemorrhage and necrosis of the liver. This necrosis appears to be identical with that seen by Gyorgy and Goldblatt.[3] It is primarily coagulative in type and centrolobular in location and is nearly always accompanied or even replaced by hemorrhage of quite variable extent in the necrotic areas. Sometimes only small periportal islets

TABLE 1
BASAL DIETS EMPLOYED IN CIRRHOSIS AND NECROSIS EXPERIMENTS

	Basal diet No 1	Basal diet No 2	Other basal diets
Casein	4 0%*	4 0*	4 0** to 18 0**
Cystine	—	0 04%	—
Salt mixture†	4 0	4 0	4 0
Cod liver oil	2 0	2 0	2 0
Vegetable oil‡	3 0	3 0	3 0
Corn starch	87 0	86 96	—
Sucrose	—	—	to 100

* Leached
** Leached and hot alcohol extracted
† Osborne and Mendel
‡ Wesson oil

of surviving liver cells remain When this picture and the hepatic cirrhosis described by us[4] occur together they appear to be essentially unrelated to each other, the hemorrhage and necrosis being superimposed on almost any phase in the development of the cirrhosis Rarely is there any evidence of the hemor

is shown in TABLE 1 It consisted of leached casein 4 per cent, Osborne and Mendel salt mixture 4 per cent cod liver oil 2 per cent Wesson oil 3 per cent, and corn starch 87 per cent A vitamin supplement, consisting of 100 micrograms of

ginal in vitamin E The most severe amino-acid deficiencies were probably of the sulfur containing cystine and methionine, although threonine was also severely limiting for growth At the time these experiments (summarized in TABLE 2) were carried out we were greatly interested in the effect of alcohol consumption of the exacerbation of the cirrhotic process For this reason this particular group of animals received, from the time they were placed on the experimental diet at weaning 20 per cent alcohol as the sole source of fluid The effect of methionine, you will note, is compared in the table in each case with that of a somewhat lower percentage of cystine This repre

As was pointed out
sions are clearly dif
els of added cystine

the incidence of cirrhosis increased while the incidence of necrosis (except at the 0 04 per cent level) decreased Choline chloride (20 mgm per rat per day) protected against cirrhosis but not against necrosis Methionine, being a precursor of both cystine and choline protected against both types of lesions when given in sufficient amounts The only exceptions to the general rule were three animals receiving choline and cystine Two of these died after 201 and 245 days respectively, on experiment and showed slight liver cirrhosis The third died after 296 days on experiment and showed hemorrhage and

TABLE 2

DIFFERENTIATION OF DIETARY DEFICIENCIES IN EXPERIMENTAL CIRRHOSIS AND NECROSIS (20% ALCOHOL AD LIB)

	No of rats	Cirr %	Nec. %	Neg %
Basal diet No 1	16	19	37	62
Basal diet No 1 + Cystine 0 04%	17	53	59	24
Basal diet No 1 + Cystine 0 04% and Choline*	10	0	60	40
Basal diet No 1 + Meth 0 05%	27	19	41	52
Basal diet No 1 + Cystine 0 08%	9	100	22	0
Basal diet No 1 + Cystine 0 16%	12	92	0	8
Basal diet No 1 + Meth 0 20%	4	0	0	100
Basal diet No 1 + Cystine 0 50%	94	90	0	10
Basal diet No 1 + Cystine 0.50% and Choline*	15	13	7	80
Basal diet No 1 + Meth 0 70%	10	0	0	100

* 20 mg choline chloride per rat per day

ecrosis It is worthy of note that 0 05 per cent of added methionine was in fficient to protect against either cirrhosis or necrosis and that 0 04 per cent added cystine appeared, if anything, to increase the incidence of necrosis as l as cirrhosis

rior to this work of ours in 1942, several important researches in the field ietary liver damage had been carried out Weichselbaum,[5] in 1935, had reported coma and death in rats deficient in cystine and methionine and had stated that post mortem examination showed definite hemorrhages throughout the liver No histological examinations appear to have been made but an excellent clinical description of the deficiency state was included in this report György and Goldblatt,[3] in 1939, published a detailed description of the lesion, and in the same year du Vigneaud and co workers[6] described hemorrhage and cell damage in the livers of rats which had received diets deficient in the sulfur containing amino acids In none of these early experiments quite naturally, can the amount of choline which the rats received either from the diet or from vitamin supplements be stated with any great degree of certainty From the descriptions of the lesions, however, it appears that no hepatic cirrhosis was seen by Weichselbaum or by du Vigneaud and associates György and Goldblatt, however stated that cirrhosis occurred with necrosis in their animals

Almost simultaneously with the publication of our differentiation between dietary cirrhosis and dietary necrosis in 1942 György and Goldblatt reported data very similar to those which we presented with, however a different interpretation of the experimental results The following year, Hock and Fink[7] described liver necrosis in rats given a diet in which yeast, in rather large amounts, served as the sole source of protein These investigators reported also that the inclusion of cystine in this yeast diet prevented the development of the hepatic necrosis This finding furnished new evidence that dietary necrosis is a separate and distinct entity from dietary cirrhosis

A most important finding not only to the differentiation of these two types of lesions but also to the whole understanding of the influence of dietary factors or their lack on the integrity of the liver, came in 1944 At this time it was

reported by Schwarz[8] that the dietary liver necrosis which he had observed in many hundreds of animals over the previous several years could be prevented by the administration of vitamin E. His method of developing liver necrosis in rats was to give them a diet containing 15 *per cent* of *alkali treated* casein as the sole source of protein. There was a high incidence of liver necrosis with no liver cirrhosis. The prevention of this lesion by vitamin E together with the more recent report by Schwarz[9] in 1951 of the existence of a third factor effective against dietary liver necrosis, strengthens still further the case for the etiological and morphological separation of the two types of liver lesions.

By 1944, then, dietary cirrhosis and dietary necrosis had been fully described, a clear differentiation of the two types of lesions had been made, both on morphological grounds and on the basis of their separation by dietary means, and diets had been described on which necrosis alone developed. In addition, it was in that year that the identification of a second factor against dietary necrosis, vitamin E, was announced.

Before leaving the historical part of this presentation, however, perhaps one additional fact should be recorded. Himsworth and Glynn,[10] in 1944 published the first of their series of papers in which they corroborated many of the findings of earlier investigators. In the same year, Glynn and Himsworth[11] described in some detail an extensive postnecrotic scarring which followed massive necrosis in some of their rats. This lesion had not been observed previously and indeed cannot easily be obtained with the diets which were in use in most laboratories at that time. It is particularly important to make this clear since Himsworth and associates appear not to have claimed the priority which is their due for this interesting observation. They appear, instead, to have credited various American pathologists with including it in the earlier descriptions of cirrhosis.

TABLES 3 to 5 show the results of some further studies in our laboratory. They were carried out in collaboration with Doctors Kenneth Endicott and Ralph Lillie who made all of the histological examinations. The basal diet and the vitamin supplement used in these experiments were the same as those

TABLE 3

DIFFERENTIATION OF DIETARY DEFICIENCIES IN EXPERIMENTAL LIVER CIRRHOSIS AND NECROSIS (WATER AD LIB.)

Basal diet No. 2		+ Choline*		+ Cystine†		+ Choline* + Cystine†	
Cirr	—	—	—	Cirr	—	—	—
Cirr	—	—	Nec	Cirr	—	—	—
—	Nec.	—	Nec	Cirr	—	—	—
Cirr	—	—	—	Cirr	—	—	—
Cirr	—	—	—	—	—	—	—
—	Nec.	—	—	Cirr	—	—	—
Cirr	Nec	—	Nec	Cirr	—	—	—
Cirr	Nec	—	Nec	Cirr	—	—	—
—	—	—	—	Cirr	—	—	—
—	Nec	—	—	—	—	—	—

* 20 mg. choline chloride per rat per day.
† Additional 0.12% cystine in diet.

TABLE 4

DIFFERENTIATION OF DIETARY DEFICIENCIES IN EXPERIMENTAL LIVER CIRRHOSIS AND NECROSIS (20% ALCOHOL AD LIB)

Basal diet No 2		+ Choline**		+ Cystine†		+ Choline** + Cystine†	
Cirr	—	—	Nec	Cirr	—	—	—
Cirr	Nec	—	—‡	Cirr	—	—	—
Cirr	—	—	Nec	Cirr	—	—	—
—	Nec.	—	Nec	Cirr	—	—	—
—	—	—	Nec	Cirr	—	—	Nec.
Cirr	Nec	—	Nec	Cirr	—	—	—
Cirr	Nec	—	Nec	Cirr	—	—	—
—	Nec	—	Nec	Cirr	Nec	—	Nec.
—	—	—	—‡	Cirr	—	—	Nec
—	Nec.	—	Nec	Cirr	—	—	—

* 20% alcohol ad lib
** 20 mg choline chloride per rat per day
† Additional 0 12% cystine in diet
‡ Died after 3 and 15 days respectively

in earlier studies except that 0 04 per cent of cystine replaced an equivalent amount of corn starch in the diet (basal diet No 2) It appeared from the previous small experiment (TABLE 2) that this level of cystine might lead to an increased incidence of hepatic lesions The findings for individual animals are presented in TABLES 3 and 4 in order to show that an occasional rat had neither type of lesion while several had both types The results in TABLE 3 clearly portray the effect of choline in suppressing cirrhosis but not necrosis, the effect of cystine, even this additional 0 12 per cent in preventing necrosis while increasing or at least not decreasing the incidence of cirrhosis, and the effect of the combination in preventing both types of lesion TABLE 4 gives the results obtained with a similar group of animals which received approximately 20 per cent of alcohol as the sole source of fluid The results are very similar to those in TABLE 3, except for the higher incidence of lesions in some groups and particularly the fact that four animals receiving the higher level of cystine, three of which were receiving choline as well, died with liver necrosis The possibility that the ingestion of alcohol may increase the incidence of this lesion probably deserves further study It is interesting that the animals receiving choline had a slightly higher incidence of necrosis than those not receiving this supplement The only animals receiving the basal diet plus choline which did not develop this lesion were two animals which died after 5 and 15 days, respectively on experiment The four animals on the basal diet which did not develop the lesion died after 42 81, 98, and 110 days, respectively on the experiment. TABLE 5 summarizes the results in TABLES 3 and 4 and permits a comparison of the findings with water and 20 per cent alcohol, respectively, as the sources of fluid.

All of these experiments were carried out with casein at a dietary level of 4 per cent as the sole source of protein In our experiments, as in those of other investigators, the development of dietary liver cirrhosis and the development of dietary liver necrosis are both dependent to a large extent on the level of protein in the diet The reason for this is clear since casein as usually

TABLE 5
SUMMARY OF TABLES 3 AND 4

	No of rats	Cirr	Nec	Neg
Basal diet No 2 (water)	10	6	5	1
Basal diet No 2 (alcohol)	10	5	6	2
Basal + Choline* (water)	10	0	4	6
Basal + Choline* (alcohol)	10	0	8	2
Basal + Cystine† (water)	10	7	0	3
Basal + Cystine† (alcohol)	10	10	1	0
Basal + Choline* and Cystine† (water)	10	0	0	10
Basal + Choline* and Cystine† (alcohol)	10	0	3	7

* 20 mg choline chloride per rat per day
† Additional 0 12% cystine in diet.

TABLE 6
EFFECT OF PROTEIN LEVEL

Casein	No of rats	Fatal kidney lesions %	Cirr %*	Nec %*	Neg %*
4%	10	0	40	20	30
6%	10	0	40	50	20
8%	12	8	82	55	0
10%	12	42	100	43	0
12%	21	24	44	25	56
14%	11	27	25	0	75
16%	11	36	0	0	100
18%	11	18	0	0	100

* Of those not dying of kidney lesions

prepared, even so-called "vitamin free" casein, has been shown by Schwarz to contain Factor 3, which prevents liver necrosis, and of course contains also cystine and methionine Both of these amino acids, as has been indicated, help to prevent the necrosis while methionine is very active in the prevention of cirrhosis TABLE 6 gives some of our data on this point In this group of experiments, the diets which were used contained leached and hot alcohol extracted casein, 4 to 18 per cent, salt mixture 4 per cent, cod liver oil 2 per cent, Wesson oil 3 per cent, and sucrose to make up the balance Vitamins were given as in the earlier experiments Water was the source of fluid A number of these animals died from the acute kidney lesions first described by

cent, no cirrhosis on levels of casein above 14 per cent, and no necrosis on levels of casein above 12 per cent In passing it might be noted that the suggestion has occurred frequently in the literature that rapid growth is essential for the development of kidney lesions Rapidity of growth is certainly a factor, but we have had no trouble in obtaining fatalities from this lesion routinely on

TABLE 7

CIRRHOSIS "SCORE" AS AFFECTED BY RIBOFLAVIN INTAKE

Riboflavin level (micrograms per rat per day)	Experiment number				
	1	2	3	4	5
5	2 0	2 3	2 3	2 0	1 6
50	1 2	1 4	1 6	1 3	1 1

Exp 1 3 No choline added to diet
Exp 4-5 25 mg choline chloride/100 g diet

diets too low in protein to permit growth, by including cystine, high levels of fat, and cholesterol in the diet

I should like to pass now to some studies on the effect of a low riboflavin intake on the development of liver cirrhosis in rats which were carried out in collaboration with Doctor George Fite These studies were prompted in part by the observations concerning "yellow liver" in dogs by Sebrell and Onstott[13] in 1938 In the light of information accumulated in large part since their experiments were carried out, it appeared possible that the condition they described might have developed as a result of combined riboflavin and choline deficiencies There were also the numerous clinical studies using diets similar to the high protein, high vitamin diet of Patek and Post[14] which seem to indicate that nutritional deficiencies other than that of choline are of importance in clinical liver cirrhosis In addition, there is the well known protection of the liver from certain toxic materials such as butter yellow[15] by an increased intake of riboflavin The diets used in these experiments contained so-called "vitamin free" casein (G B I) 10 per cent, a modified Osborne and Mendel salt mixture* 4 per cent, cod liver oil 2 per cent, Wesson oil 3 per cent, and sucrose 81 per cent The diet of groups 4 and 5 in TABLE 7 contained also 25 mgm per cent of choline chloride Two vitamin supplementations were used, the one which I have previously described and another which differed only in that the riboflavin level was reduced from 50 mg to 5 mg per rat per day At weaning, Osborne and Mendel rats were divided equally on a litter and sex basis and maintained under the experimental conditions described for one year unless death intervened The seven groups in the table represent a total of 176 rats Following death or sacrifice of the animals, histological examinations were made by Doctor Fite Each liver was given a score, 0 if no cirrhosis could be observed, or 1, 2, or 3, if cirrhosis was observed, the number assigned depending on the severity These figures were then averaged for the group The higher figures in the table, therefore, represent the greater average incidence and severity of cirrhosis It appears that the animals which received the 5 mgm level of riboflavin in all experiments had a greater degree of cirrhosis than their litter mates which received 50 mgm of this vitamin daily The probability of such an outcome due to chance alone in 5 consecutive experiments is 1 in 32 The result is significant at the 03 level On the

* Prepared according to the directions of Osborne and Mendel except that the sodium fluoride is reduced to 1 per cent of their level and 0.313 gm of $CuSO_4 \cdot 5H_2O$ (equivalent to 0 2 g anhydrous $CuSO_4$) is added

TABLE 8

EFFECT OF COMPOSITION OF FAT COMPONENTS OF DIET ON DEVELOPMENT OF KIDNEY LESIONS

	No of rats	Acute lesions	Healing or scars	Neg
		%	%	%
Stearic acid	67	52	24	24
Palmitic acid	49	39	20	41
Oleic acid	48	0	17	83
Soybean oil	8	0	0	100
Soybean flakes	8	37	25	37

basis of these results we should like to propose that the level of riboflavin in a diet, not completely adequate in other factors, may influence the development of liver cirrhosis

Finally, I should like to present the results of some experiments on the effect of the composition of the fat component of the diet on the development of fatty livers and on the development of kidney hemorrhage and necrosis These studies were carried out in collaboration with Doctor Kenneth Endicott who made all of the histological examinations The general plan of the studies was for weanling Wistar or Sprague Dawley rats to be divided according to sex and litter and given one of three experimental diets These diets contained casein, which had been leached and extracted with both cold and hot alcohol 4 per cent, modified Osborne and Mendel salt mixture 4 per cent, cystine 0 5 per cent, sucrose 81 5 per cent, and palmitic, stearic, or oleic acid 10 per cent In a small experiment covered by the last two lines in TABLE 8, soybean oil, or soybean flakes which were prepared by the hydrogenation of this particular lot of soybean oil, was substituted at the same level for the 10 per cent of fatty acid Both sexes of rats were used, the ratio in each group being roughly three males to one female In the case of 36 animals, 12 from each of the first three groups, the experiment was terminated after 10 to 14 days and the livers were analyzed for total fat In the case of 35 additional animals in these three groups, the experiment was terminated by sacrifice of the animals at 16 to 21 days The remaining 93 rats divided between the three groups were kept on these three diets until their deaths which occurred after experimental periods ranging from 11 days to 216 days As may be seen from the table, the incidence of acute kidney lesions ranged from 0 per cent for those receiving oleic acid to 39 per cent for those receiving palmitic acid and 52 per cent for those receiving stearic acid Were we to exclude the animals which were sacrificed, the results would be even more striking None of 22 animals on oleic acid, 52 per cent of 29 animals on palmitic acid, and 69 per cent of 42 animals on stearic acid showed acute lesions when the experiments were permitted to proceed to the death of the animal It will be noted further that the results of a small experiment, carried out with unhydrogenated soybean oil and with the same lot of oil hydrogenated to a form known as soybean flakes, support the idea that the degree of saturation of the fat or fatty acid which makes up the fat portion of the diet influences very greatly the incidence of this fatal acute

Table 9

Liver Fat as Affected by Nature of Fatty Acid Ingested

	No of rats	Fat	
		%	Range
Palmitic	9	25 4	(12 0–38 0)
Stearic	9	18 3	(13 4–27 9)
Oleic	9	16 2	(11 2–25 0)

choline-deficiency syndrome Table 9 gives the fat analyses of the livers of nine groups of litter mates There appears to be little difference in the fat content of the livers of the rats receiving oleic or stearic acid The level of liver fat of the rats which received palmitic acid appears to have been somewhat higher

An insufficient number of the 93 animals survived the acute kidney lesions in this series of experiments to permit us to draw any definite conclusions as to the incidence and severity of cirrhosis I shall merely mention that of the animals thus surviving one out of 13 or 8 per cent on stearic acid, three of 14 or 21 per cent on palmitic acid, and six of 22 or 27 per cent on oleic acid were found to have some degree of cirrhosis The oleic acid animals had not only the highest incidence of cirrhosis but also showed the more severe lesions These fatty acids, as might have been expected from our earlier work,[16] appeared to be very poor precursors of ceroid since even in those livers which were cirrhotic there was either none of this pigment or only a trace One rat receiving palmitic acid and one receiving oleic acid showed massive liver necrosis

In conclusion, we should like to suggest on the basis of these results that the nature of the fatty acids in the fat component of the diet deserves consideration in studies of fatty livers acute choline deficiency, and hepatic cirrhosis. It will be of interest to determine whether or not the nature of the fat component of the diet is of importance in the development of liver necrosis as well

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Discussion of the Paper

Doctor Paul Gyorgy (*School of Medicine, University of Pennsylvania Philadelphia Pa*) The distinction of acute hepatic necrosis from chronic fibrosis, as first proposed by Doctor Daft and Doctor Sebrell, has proved to be a very useful and fruitful concept In experimental hepatic injury in rats, the etiologic dietary factors for necrosis and cirrhosis appear to be definitely different In our own laboratory, we have observed independently of Doctor Daft and Doctor Sebrell the beneficial effect of cystine and methionine in experimental necrosis of the liver and the injurious effect of cystine regarding cirrhosis Nevertheless we have abstained from a clear cut separation of necrosis from cirrhosis for two reasons (1) In toxic hepatic injury, the hepatotoxin in large doses may produce necrosis and, in small repeated doses typical Laennec's cirrhosis, and (2) Cystine in amounts not much in excess of the therapeutic dose range will not only prevent but produce hepatic necrosis

Later, it has been found, first by Schwarz, then independently in our laboratory, that hepatic necrosis is preventable not only by sulfur-containing amino-acids but also by vitamin E This raised the question whether necrosis is a simple or conditioned deficiency

THE SEQUENCE OF PATHOLOGIC EVENTS IN THE DEVELOPMENT OF EXPERIMENTAL FATTY LIVER AND CIRRHOSIS*

By W. Stanley Hartroft

Banting and Best Department of Medical Research, University of Toronto, Canada

Introduction

Interest in the fatty livers which may be produced in experimental animals by feeding diets low in choline and its precursors (betaine, methionine and methionine-containing protein) has extended in our laboratories at Toronto over a period which can be said to have originated with the discovery of insulin in 1922 and has continued up to the present time. The sequence of pathologic events which begins almost as soon as an animal is placed on the low-choline regimen has now been studied in a large number of animals. Most of the early studies which resulted in the discovery by Best and his associates[2] of the lipotropic action of choline were carried out on dogs, but since the end of World War II most of our experiments concerning the pathological changes in choline-deficient animals have been carried out on the rat. Other species of animals are similarly affected as will be reviewed by Professor Best and his co-workers in the next paper. Much of the available evidence indicates that man should probably be included in the list of animals susceptible to some of the pathologic effects of low choline diets.

Some of the early morphological changes which affect the liver in dietary choline deficiency are those described by MacLean and Best in 1934.[15] In this paper were reported the appearances of livers of dogs fed diets low in choline and of those of control animals which had received the same basal diet supplemented with adequate amounts of the lipotropic agents. Photomicrographs were published in black and white as well as colored drawings of some of the microsections. Detailed protocols of some of the animals were published in full. In this early paper are several features worthy of special mention here (FIGURES 1 and 2). The *intracellular* storage of fat within parenchymal liver cells is well illustrated and described. In addition, these photomicrographs show definite evidence of the *extracellular* phase of fat storage within what we now call fatty cysts. Furthermore, in two of the illustrations there is evidence of early fibrosis in these livers and in the protocols it is expressly stated (Dog 1) that in some areas of the liver the number of fibroblasts and leucocytes in these areas was abnormally large and that the liver cords, however, were separated by dilated capillaries and by areas of beginning fibrosis. These reports clearly foreshadowed the recognition four years later of cirrhosis in animals fed low choline diets by Chaikoff and Connor[4] in dogs and by György and Goldblatt in rats.[8]

We have begun with this description because many phases in the sequence of events which we are about to describe in the livers of choline-deficient rats were already evident in these earlier studies. In rats similar phenomena

* Much of the work reviewed in this paper was supported by the National Cancer Institute of

can be studied in a greater number of animals because the lesions make their appearance within shorter periods than is the case in dogs. All the findings to be set forth briefly below have been previously published[7, 8, 9, 11, 1] or are in press.[10] This paper will consist, therefore, of a summation of our conclusions based on these studies. Data concerning dietary methods, histological techniques and photomicrographic evidence are given in full in the publications mentioned.

1. Intracellular phase of the accumulation of abnormal fat in livers of choline deficient rats. "Intracellular lipohepatosis"

Within a few hours after allowing rats access to diets low in choline, stainable fat appears *within* many of the liver cells.[5] The fat is clearly intracellular in position and, at this stage, is seen in greatest amount within parenchymal

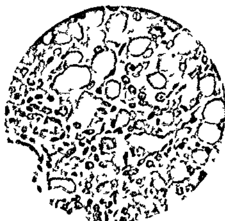


FIGURE 1. Compare with FIGURE 2. Early fibrosis in dogs resulting from atrophy and fibrotic replacement of fatty cysts. Figure 5B in MacLean and Best.[6]

cells in centrolobular and nonportal regions. Stainable fat droplets may not accumulate in portal liver tissue until a later stage. Even such an early manifestation of the hepatic lesions of choline deficiency is in accord with the path onomy that the effects of this dietary abnormality are always first observed and are present in greatest degree in the nonportal regions of the liver. This inciple, as we shall see, holds true at all stages, including those of advanced rrhosis.

Many terms have been used in the past to describe the abnormal accumula on of fat in the livers of choline deficient animals. Such have included fatty iange, fatty infiltration and fatty degeneration. In our opinion, none of iese phrases is free from ambiguity when used to describe the lesions in epatic hypocholinopathy. Fatty change might imply merely a change in the ate of the liver lipids, but we know[1] that the fatty droplets represent an cumulation of excess fat brought to the liver from the ingested food. The

fat is initially held within liver cells and not in adipose tissue cells between them as the term fatty infiltration would [illegible]

[illegible]

[illegible] we suggest the term

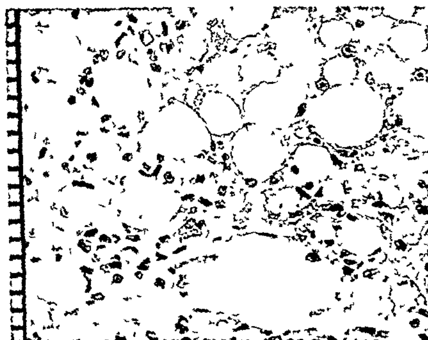


Fig. [illegible] Region [illegible] central [illegible] stage of [illegible] undergoing [illegible] Paraffin section hematoxylin and eosin [illegible]

lip hepatosis for this condition The phase described in this section is that of *intracellular lip hepatosis* This is followed by *extracellular lip hepatosis* of which the [illegible] final feature is the formation of fatty cysts

Extracellular lip hepatosis [illegible]

In the [illegible]
in rats fat [illegible]
the [illegible]
degree in the [illegible] which thus escapes from within [illegible] surrounded by the latter and is therefore contained as relatively large pools of lipid within simple epithelial cysts The cysts are

formed in the following manner Progressive enlargement of fat globules within neighboring liver cells compresses the adjacent intervening segments of their limiting cell membranes (which have been caught between the globules) into morphologically single tenuous septa By its formation such a septum conjoins the parent cells but still separates the adjacent fat globules and prevents them from coalescing If the globules continue to enlarge they eventually so stretch the interposed septum that it is torn As a result, the fat globules in the torn cells coalesce The resultant pool of fat is enclosed however, by the crescentic remnants of the cytoplasm of the parent group of cells which are still linked together The nuclei of the cells appear essentially unaltered desp te the fact that the l m t ng m mb

T

re

tissue but is contained within a simple, epithelial cyst Fatty cysts once

pathological routes to be described When fat is no longer either intracellular or intracystic, it appears to constitute a grave menace to the health of the animal

3 Extracellular lipohepatosis rupture of fatty cysts and formation of trabeculae

It has been observed repeatedly that the distribution of fatty cysts in the livers of choline deficient rats presages that of the fibrous trabeculae which are formed later This juxtaposition is not merely fortuitous for the evidence clearly indicates that it is the dissolution of ruptured cysts that produces the trabeculae Fatty cysts form initially as already mentioned around the centrolobular radicles of the hepatic vein From here they extend in annular chains which link adjacent central veins They apparently follow this course by virtue of the fact that if they extend into any other portion of the lobular parenchyma they will be advancing more or less directly towards nearby portal areas The progressive sites in which fibrosis develops are identical with those in which cysts previously have formed Trabeculae result from condensation of the parenchymal and stromal remnants of ruptured fatty cysts This condensation of cyst remnants occurs whenever the cysts lose their fatty contents Rupture of cysts over 100 micra in diameter (as measured in paraffin sections) is apparently nearly always accompanied by escape of fat Large cysts are covered with a fine network formed of bile capillaries and sinusoids which originally ran between the liver cells that have taken part in the formation of the cyst wall The larger the cyst the more extensive this network, and accordingly the more likely that some component of the latter will be torn with rupture of the cyst wall As a result free communications may be established between the lumen of the cyst and those of sinusoids or bile ductules Fat may be drained from the cyst by either of these two routes, and the remnants of the emptied structure condense into a fibrotic residuum In this manner the annular chains of cysts connecting adjacent centrolobular regions progress

into the fibrous trabeculae familiar to anyone who has observed early stages of experimental dietary cirrhosis in animals

4 The development of a "portal" distribution of the fibrous tissue

In their initial reports Gyorgy and Goldblatt[8] interpreted the distribution of fibrosis in choline deficient rats as portal. They were studying late stages of the condition. Although it is not clear from the work of Lillie *et al.*,[11] Glenn *et al.*,[4] and from the foregoing description that in the early stages, the trabeculae are nonportal, later fibrosis clearly develops around large, easily identified portal triads. In this sense, the cirrhosis is unquestionably portal in distribution. But the fibrosis in these sites replaces parenchyma which although adjacent to *conducting* portal vessels (Elias) is not periportal in the sense that it is supplied by blood which has *not* previously come into intimate contact with liver cells. Parenchyma that lies beside these large conducting veins is supplied by sinusoids which do not frequently or necessarily communicate directly with the near by veins. In most instances it has been shown that these sinusoids receive their blood via others that in turn communicate with terminal venules, which actually perform the function of distributing the blood to the liver parenchyma. The large conducting veins are those which bring the blood to the terminal venules, and the latter empty the blood into sinusoids which rarely communicate directly with vessels of the order of size of veins. This is generally true of tissues and organs throughout the entire body, for example in the kidney and pancreas; capillaries and sinusoids nearly always arise from venules rather than directly from veins. Portal venules lie in the center of a structural unit of liver tissue described by Rappaport and his co-workers as the *hepatic acinus*.[10] The periphery of an acinus may sometimes lie against a conducting vein and hence parenchyma in such a site, although periportal in a geographic sense, receives its blood via other sinusoids from a terminal venule. Such sites are, in terms of the entry of fresh blood into a hepatic unit, no more functionally periportal than are sites which are obviously closer to radicles of the hepatic vein. Fatty cysts and subsequently their trabecular remnants may therefore appear in these regions adjacent to conducting veins. Fibrosis in such positions at first appeared to constitute a paradoxical exception to the pathognomy that nonportal regions of the liver are those most severely affected in animals suffering from a dietary deficiency of choline. If the hepatic architecture is interpreted according to Rappaport's concept of the hepatic acinus, and in terms of the functional entry of fresh blood into sinusoids around terminal distributing venules, the paradox is resolved. It then becomes clear why initially nonportal lesions lies one geographically (only) periportal and surround large conducting portal triads. The latter structures are prominent even under the lower powers of the microscope and, unless the sites of the smaller, relatively inconspicuous terminal venules are examined with the aid of higher magnifications, it may not be realized that the adjacent parenchyma (which is both geographically and functionally periportal) is free from fibrosis. The term "portal cirrhosis" is acceptable at this stage from a purely descriptive standpoint, but it should be

realized that the sites of fibrosis are actually nonportal with reference to bloo supply In microsections of cirrhotic livers obtained at autopsy from alcoholi patients we have observed that the parenchyma around terminal porta venules is usually quite free of fibrotic lesions,[10] although the large conductin veins *in these cases frequently were surrounded by pathological amounts o* fibrous tissue This suggests that the distribution of the trabeculae in al coholic cirrhosis in man is also periportal only in a geographic sense and that in this respect as well as perhaps in others dietary cirrhosis in choline deficien rats is the morphologic counterpart of that found in alcoholic man

5 *Nodular hyperplasia*

After four or five months of choline deficiency, focal areas of hyperplasi throughout the liver become prominent features in microsections In thes areas which surround the terminal portal venules, mitotic figures may be founc with ease Frequently, cells with two three, or even four nuclei may be en countered As a result of this proliferation nodules of compensatory paren chymal tissue are formed and they project, on the surface of the organ thu producing the characteristic hobnail appearance which is such a striking featur of these livers when examined grossly Growth of the nodules results in com



FIGURE 3 Advanced hyperplasia of bile ducts and regenerating liver cells in choline deficient rat's liver in stage of late nodular cirrhosis This field may represent precancerous or early neoplastic change Paraffin section hematoxylin and eosin stain Divisions of scale are 10 micra apart

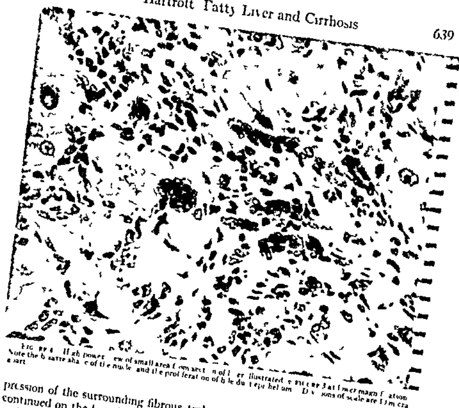


FIGURE 4. High power view of small area from section of liver illustrated in FIGURE 3 at lower magnification. Note the bizarre shape of the nuclei and the proliferation of bile duct epithelium. Divisions of scale are 10 micra apart.

pression of the surrounding fibrous trabeculae. Even though the animals are continued on the low choline regimen, the newly formed tissue rarely becomes as fatty as that previously present. In the regions of compensatory proliferation the terminal vessels may lengthen and divide. Studies of the arterial supply of the large nodules have not yet been made.

6. *Neoplasia*

At the edges of the nodules, as well as within their substances in some instances, isolated liver cells may assume bizarre forms with rather wide variations exhibited in their size, shape and staining reactions (FIGURES 3 and 4). In our experiments this appearance rarely suggests anything more than very active hyperplasia. There is, however, little doubt that in susceptible strains of rats, cirrhosis induced by low-choline diets may progress to true neoplasia. The evidence for hepatic tumor formation in choline deficient rats is presented in a paper by Doctors Copeland and Salmon who first reported its occurrence.[4]

Conclusions

The escape of fat from cysts into the vascular system results in intermittent or chronic episodes of fat embolism. These emboli are carried to the heart

lungs, and kidneys where microscopic evidence of their damaging effect has been noted[8, 9] Other organs may well be involved in this phenomenon, and it is possible that the arteriosclerotic changes in the aorta, carotid arteries, and coronary vessels which we have reported in choline deficient rats[12] may be another result of fat circulating in the blood stream in this obviously pathologic state

We have followed the regressive changes which regularly develop in fatty hepatic cysts of previously choline deficient rats when lipotropic factors are restored to the animals' diets[13] Intracellular fat is mobilized within a matter of hours, thus rendering the cysts particularly prominent Weeks, and, in some instances, months, of choline administration may be required before the fat within cysts is absorbed by the cells forming their walls Once lipid regains this intracellular position, dietary choline rapidly effects its disappearance The fact that cysts may remain for relatively long periods in previously fatty livers even after therapeutic measures have removed all intracellular, stainable fat offers a diagnostic aid in the identification of fibrosis of those types which may have been initiated by lipohepatosis We have examined sections from one hundred consecutive, unselected cases of cirrhosis coming to autopsy in the Department of Pathology at the University of Toronto, in an effort to evaluate the possible diagnostic significance of residual cysts within fibrous trabeculae Almost without exception, these intratrabecular cysts were present in cases of cirrhosis in which the history clearly indicated that they had been associated at some time with lipohepatosis[9]

Fatty cysts are the cytometaplastic links between lipohepatosis and cirrhosis In miniature

findings in livers of

increasingly fatty, fil

formation and rupture of fatty cysts is by condensation of their remnants, the direct cause of fibrosis As the livers become increasingly fibrotic their total fat content, as assayed biochemically decreases This phenomenon is explained by the concept that the formation of the fibrous trabeculae is dependent on the escape of fat from the cysts and thus eventually from the liver via either the biliary or vascular systems These events would lead one to expect the total fat content of the liver to vary inversely with the amount of fibrous tissue as is the case This relationship is probably emphasized by the fact that new tissue in regeneration nodules rarely undergoes appreciable fatty change The concept of the formation and natural history of fatty cysts permits an appreciation of the pathogenic relation between excessive fat and fibrosis in livers Formation of fatty cysts and condensation of their ruptured remnants into fibrous trabeculae implies *ipso facto* that the fibrosis is the direct result of overloading focal regions of the liver parenchyma with abnormal fat It is apparent from this that the mere presence of even appreciable amounts of fat in the liver will not necessarily lead to fibrosis Fibrosis will result only if the accumulation of fat is so great that rupture of cells produces cysts which in turn, disintegrate This explanation may serve to help resolve some of the apparently conflicting conclusions which have been previously drawn concerning the cirrhogenic nature of lipohepatosis

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Discussion of the Paper

Doctor I N Dubin (*Armed Forces Institute of Pathology Washington D C*) In the Registry of Hepatic Pathology we have had the opportunity of seeing a large number of cases of human liver disease studied by needle biopsy and on the basis of this experience we have been led to conclusions which are somewhat at variance with those of Doctor Hartroft We have now seen biopsy material from over 400 cases of portal cirrhosis (*i e*, so-called nutritional or alcoholic cirrhosis) and from a similar number of cases of fatty metamorphosis with or without fibrosis We should summarize our experience as follows

(1) With rare exceptions, we have not observed "fatty cysts" in man comparable to those described in rats by Doctor Hartroft. When present, the cysts were minute, extremely few in number and not related to fibrosis

(2) On the contrary we are of the opinion that fatty metamorphosis of itself does not lead to hepatic necrosis or cirrhosis We have seen cases of fatty

liver studied by serial biopsies which at the end of two or three years still failed to show significant portal fibrosis let alone cirrhosis

(3) In our experience, fatty metamorphosis of the liver in any one patient may come and go without appreciably *affecting the degree of portal fibrosis or cirrhosis*

factors which causes the necrosis also happens to cause fatty metamorphosis Fatty metamorphosis without the basic factor of liver necrosis does not lead to fibrosis On the other hand, we have seen extensive necrosis of liver without appreciable fatty metamorphosis yet this will be followed by cirrhosis

This type of cirrhosis comes about as the result of two main processes increase of collagen and nodular regeneration of liver cells (a) The diffuse necrosis of liver leads to a fairly diffuse but somewhat irregularly distributed increase in collagen not only in portal areas but particularly in the parenchyma which it traverses in thin bands (b) In the absence of the original reticular *framework to guide the regenerating liver cells the newly formed parenchyma* grows in the form of roughly spherical nodules without proper relationship to pre existing key structures such as portal areas and central veins Indeed the intralobular bands of collagen may be partly responsible for this abnormal architecture by forming a new but haphazard framework along which the regenerating tissue grows

(5) The acute necrotizing phase is characterized by a fairly diffuse degeneration and necrosis of liver cells many of which are transformed into Mallory bodies Accompanying this is a moderate amount of inflammatory reaction scattered throughout the lobules and involving portal areas as well Fatty metamorphosis may or may not be present At this point three things may

Doctors Hartroft and Dubin Studies with Doctor Hans Elias based on three *dimensional reconstruction* and geometrical analysis have convinced me that there are several pathways along which fatty liver may proceed to cirrhosis *formation One pathway is that which Doctor Hartroft has explored by his* thorough and ingenious investigations I feel however that this occurs

~ sts are found

the marked

rosis results

from collapse of the fatty cysts or from the formation of collagen fibers in the vicinity of the cysts The second pathway is the result of necrotizing and inflammatory changes complicating the fatty liver, somewhat similar to the

processes which Doctor Dubin just described so well The inflammation or the necrosis with associated inflammation leads to an irregular fibrosis with the formation of collagen fibers in the parenchyma Moreover, radiating or stellate fibrosis of the portal triads develops The latter may possibly be due to irritation by breakdown products drained from the parenchyma to the portal triads In our opinion, however, the most important pathway results from stress within the parenchyma exerted from the vicinity of regeneratory, ordinarily fat free, liver cell plates, usually several cells thick, and the fat containing one cell thick plates This stress produces planes of breakage in which fibers develop to form a straight septum, subdividing the lobule In human fatty livers, where periods of nutritional disturbance alternate with at least partial recovery, these stress planes probably develop more readily In conclusion, I believe that the degree of participation of the different pathways varies in the individual cases This accounts for the polymorphic picture seen in the development of cirrhosis from the fatty liver in man

DOCTOR CHARLES S DAVIDSON (*Thorndike Memorial Laboratory, Boston City Hospital, Boston, Mass*) In studying alcoholics with acute liver disease at the Boston City Hospital, we have observed ubiquitous fatty changes in the livers but we are not convinced that the fatty changes always constitute the most important pathological abnormality A typical hepatic lesion characterized by parenchymal disorganization, intracellular hyalin and necrosis frequently occurs and is distinct from the fatty lesion This lesion correlates better than does fat with the acute severe liver impairment which often leads to fatal coma The lesion seems to have a predilection for females A summary of this work done in collaboration with Doctor Gerald G Phillips Dante Campagna Pinto, and Frederick Parker Jr, was published in the Proceedings of the American Federation for Clinical Research meeting for May 1953

DOCTOR HARTROFT In reply to Doctor Dubin's comment I should like to emphasize the following points We have examined nearly one hundred cases of cirrhosis in human patients and in a very high percentage of cases associated with alcoholism diabetes obesity and anemia fatty cysts in various stages were found *within* fibrous trabeculae Accordingly we have named cysts in this position *intratrabecular fatty cysts* and from their position have concluded that as in the rat these cysts may be cirrhogenic in nature These results have already been published (Hartroft 1953 Arch Path 55: 63 69)

Doctor Dubin is of the opinion that fatty metamorphosis of itself does not lead to hepatic necrosis or cirrhosis and cites instances of fatty livers which he has followed by serial biopsies and which at the end of two or three years, failed to show fibrosis or cirrhosis We have never maintained that an abnormal accumulation of fat leads to necrosis, nor have we ever maintained that the mere presence of stainable fat in the liver leads to fibrosis All our studies both experimental and clinical of the pathogenesis of dietary cirrhosis lead us to believe that not only must fat be present in the liver to produce fibrosis but also it must be accumulating so rapidly and to such a degree that it ruptures cells thus leading to parenchymal damage The complete history of fatty

cysts their formation, rupture, atrophy, and condensation of stromal remnants into fibrous bands, describes a series of steps by which excessive accumulation of hepatic fat leads to traumatic rupture of distended cells and to fibrosis. We have seen many instances in rats where the conditions were such that the lipotropic deficiency, although severe enough to cause accumulation of fat in liver cells, was not sufficiently extreme to produce fatty cysts and their sequelae, including fibrosis and cirrhosis It is equally true that, once cirrhosis is established similar degrees of "nontraumatic" accumulation of fat could come and go, as Doctor Dubin states, without appreciably affecting the degree of cirrhosis

In the experimental animal subjected to dietary choline deficiency (uncomplicated by a concomitant deficiency of tocopherol, cystine, and methionine), there is no doubt whatsoever that a cirrhosis results In these animals, the consensus of opinion of those experimental pathologists who have studied the livers in detail is that necrosis of parenchymal cells is hardly ever seen *We are using the term necrosis here in the sense meaning acute or sudden death* of tissue It is apparent, however, that the slow death of cells or atrophy, associated with rupture of fatty cysts is, in a sense, necrosis, if the term is used with this broad meaning We prefer, however, for the sake of clarity, to reserve the term necrosis for the sudden acute type of cell death charac

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Doctor Popper has excellently summarized the points regarding the patho-

In all attempts to correlate the amount of fatty change with the amount of fibrosis and/or cirrhosis in sections whether of the experimental animal or alcoholic man, it is well to emphasize that there is abundant evidence that the greater the fibrosis the less the amount of fat This inverse correlation is again reflected by the "life story" of fatty cysts seen in livers of choline deficient rats The more cysts which rupture, thus allowing their contained fat to escape from the liver by one or more of the pathological routes we have described (Hartroft W S & J H Ridout 1951 Am J Path 27: 951–991) the greater the fibrosis resulting from their condensed parenchymal and

and cirrhosis If in addition the animal becomes sick and fails to eat for a period before death or if it is treated with adequate diet and lipotropic factors much of the fat in the cirrhotic liver will be mobilized (Hartroft & Sellers 1952 Am J Path 28 387–399) Residual intratrabecular cysts may not be numerous and almost certainly will be small, but in the experimental

animal where every stage of the fat accumulation and subsequent cirrhosis has been followed, it is clear that these few persistent cysts represent the stigmata of a liver that was previously fatty to the "bursting point." Similar findings in man may have the same significance and may, therefore, provide important diagnostic evidence suggesting the etiology and pathogenesis of the cirrhosis manifest at autopsy. We believe that, if these points are kept in mind, much of the apparent confusion concerning the pathogenic relationship of fatty to cirrhotic livers might be resolved, not only in experimental animals, but also in man, including perhaps some of Doctor Davidson's cases.

THE LIPOTROPIC FACTORS

By C H Best, C C Lucas, and Jessie H Ridout
Banting and Best Department of Medical Research, University of Toronto, Canada

The nutritional role of choline was discovered[1, 2] in 1932, so that this year it comes of age as a dietary factor Choline was the first of the accessory food factors which was shown to protect the liver, *i e*, in its absence from the diet, pathological changes appear promptly in the hepatic cells The work on choline, as a survey of the literature clearly shows, stimulated great interest in liver fat in the effect of large accumulations of fat on hepatic function, and in the part played by other dietary factors in the protection of the liver

The very general and broad title assigned to us here has made us somewhat uncertain about what is desired The topics to be dealt with by our learned colleagues on this comprehensive program leave us but little in the way of definite responsibility, and we are therefore quite free to discuss certain general aspects of our subject

We are all familiar with the fact that choline, betaine, methionine and under some conditions, vitamin B_{12} and inositol, protect rats in varying degrees against the development of fatty livers In the absence of choline from the diet, many species of animals have been found to develop this abnormality The rat,[1, 2] dog,[3, 4, 5, 6] mouse[3, 7] rabbit,[8] hamster,[9] calf,[10] pig,[11] and duckling[12] exhibit fatty livers when the diet lacks sufficient of the lipotropic agents choline or its precursors Some of the classical lesions are illustrated in Professor Hartroft's presentation (page 633) In some species, such as the guinea pig, it is very difficult to produce an abnormal deposition of fat in the liver by feeding diets low in choline[13] We must always keep in mind that interference with the function of hepatic cells by means other than deficiency of the lipotropes may lead to fatty livers Fatty livers of guinea pigs on scorbutic diets[14] are probably not attributable to a lack of dietary choline

similar renal lesions have been reported in young pigs[16] and calves[10] The failure to produce this kidney lesion in young mice and puppies, both of which develop fatty livers readily enough, reveals a problem which may possibly require extended study A comprehensive investigation and comparison of the relevant enzyme systems will undoubtedly help An interesting sequel to the renal lesion in rats is the appearance in later life of hypertension[17] This has not yet been reported in other species You are also familiar with the failure of baby chicks,[18] turkey poults[19] and ducklings[20, 21] to grow and their tendency to develop slipped tendon disease on choline deficient diets In our early studies on the effects of choline in preventing and curing the pathological changes in the diabetic dog, we mentioned "areas of beginning fibrosis" which did not appear to be as favorably affected by choline as the fatty changes[22]

but we did not observe cirrhosis until after the work of Chaikoff and Connor[23] on dogs and György and Goldblatt[24] on rats. The role of choline deficiency in the production of fatty livers and cirrhosis in man is less clear cut. The crucial experiment to determine the lipotropic activity of choline in man has not yet been performed. The nearest approach is that of Phillips, Gabuzda, and Davidson.[25]

By these brief references to the effects of choline and its precursors in various species, we have been leading up to reconsideration of the word "lipotropic." What should be included under this term? The word was introduced to describe substances which decrease the rate of deposition and accelerate the rate of removal of excess liver fat and was so defined by Best, Huntsman, and Ridout.[26] While the word *dietary* was not specifically mentioned, it was obvious from the context that the active principle under discussion was a component of the diet. The word lipotropic has been carelessly used, but some of the misuse is perhaps due to real difficulties in deciding on the proper scope of the term. There is, of course, no justification whatever for many of the misapplications, *e.g.*, a change in lipid content of a fat laden pterygium on the cornea or in the amount of depot fat in obese human subjects or in animals. The term lipotropic is not applicable to changes in lipid concentration in the blood or generally in the body. The word does not apply to *increases* in liver fat, which at least one writer has suggested. The original definition stated that the term refers to *decreases* in fat deposition and is applied to changes in the *liver*. At one time, however, we[27] suggested an extension of this to include the other established effects of choline and its precursors, *viz.*, prevention of renal lesions and of cirrhosis. Whether this proposal was wise has since been debated many times in our laboratory. Some of these changes may include effects involving fat deposition, *e.g.*, in the kidneys[28] or perhaps in the walls of blood vessels. If it is firmly established that the primary, or a highly significant part of the process initiated by choline in organs other than the liver is essentially similar to that in the hepatic cells, *i.e.*, involving the prevention of excess fattiness. The term lipotropic may then be extended to include these other lesions.

Let us compare the antiperotic action of choline and its effect in preventing the renal hemorrhagic syndrome. We can find no justification for calling the antiperotic effect lipotropic, but if prevention of deposition of excess fat in the proximal renal tubules is the essential process, this protection of the kidney may be considered a lipotropic phenomenon.

On the other hand, although the growth effect of choline, which can be clearly demonstrated under certain conditions, is mediated through biochemical processes intimately interrelated with lipotropic action, this action should be designated by a term other than lipotropic.

We should stress here that a substance may exert a lipotropic effect without being a lipotropic agent. It might increase the rate of formation of, or potentiate in some way the action of, a lipotropic agent.

The role of antibiotics, such as aureomycin, in lipotropic phenomena is not too clear. Doctor György will discuss these in detail. We feel that they have

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not qualified as lipotropic agents although they do modify the requirement for choline and methionine Thiamine, calcium, vitamin A or any other dietary essential, in whose absence the food intake and rate of growth are affected, may also affect the choline requirement We cannot call all the essential food factors antilipotropic agents The lipotropic effect of the antibiotics cannot be due to change in food intake as Gyorgy has shown, but there is still a possibility that a change in absorption may play a role The consideration of the antibiotics later in this monograph is of pertinent interest

To continue our discussion of the term lipotropic, since a fatty liver is always a forerunner of the cirrhosis of choline deficiency, one can properly call the *preventive* effect of choline a lipotropic effect Can one say, however, that the curative action of choline on a fatty cirrhotic liver is partly or wholly lipotropic? It would appear certain that a part of the reticulum or fibrotic tissue actually disappears as an end result of choline action, but it can not be stated at present whether this result is secondary to an effect on fat to the regeneration of hepatic cells which the disappearance of fat may make possible or to a direct attack on the fibrous tissue

Another interesting question is raised by the finding [9, 30] that, under certain conditions large accumulations of fat may persist for long periods in the liver without the appearance of fibrosis In our own experience this has been observed in experiments in which vitamin B_{12} prevented the development of cirrhosis This effect of vitamin B_{12} was first reported from Gyorgy's laboratory, but the details of this particular aspect of their studies have not yet been published Doctor Drill will be discussing the role of vitamin B_{12} in lipotropic phenomena We should like to mention however, our recent finding that rats may be maintained with vitamin B_{12} on low choline diets (9 per cent protein and very low in methionine) for 16 months without cirrhosis but with [illegible] (12 to 15 per cent) In 22 [illegible]

Gyorgy's[31] original report This raises the question does vitamin B_{12} specifically prevent the cirrhosis, or does it act by increasing the biosynthesis of [illegible]

be that vitamin B_{12} acts by increasing the rate of turnover rather than merely reducing the amount of liver fat?

In confirmation of Salmon Copeland and their colleagues [32, 33, 34] we have seen a dramatic effect of vitamin B_{12} on the renal lesions in young rats yet in others on similar diets with a lower 'lipotropic background no beneficial effect whatever from B_{12} was observed Similarly, in older rats others have demonstrated that by alteration in the dietary background one may either reveal or fail to elicit the lipotropic effect of vitamin B_{12} [31, 35, 36, 37, 38]

In attempting to account for these observations we have all been forced to recognize the importance of several nutritional dilemmas The first of these has been appreciated for some time and will be discussed more fully later,

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to supply the essential amino acids without choline precursors is a problem
of the highest order While it is true that no proteins free from methionine
are known that are readily available in quantity, the major d
nutritional dilemma rather than the question
contains a labile meth
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to loss of appetite I known also that when the caloric intake is low, even
severely hypolipotropic diets do not produce fatty livers Thus a diet *low* in
methionine seems more suitable for lipotropic studies than one completely free
from it

Two chemical procedures are available for eliminating methionine from proteins Welch[44] hydrolyzed casein with hydriodic acid to demethylate the methionine After removal of most of the hydriodic acid by evaporation *in vacuo* and of the rest with silver carbonate, the remaining amino acids were obtained for feeding by evaporation of the solution The second chemical method of obtaining a diet free from methionine, devised by Toennies[45] depends upon the fact that hydrogen peroxide converts methionine, even in the intact protein, to the sulphone which is inert nutritionally

To prepare diets low in methionine one may utilize the proteins of peanuts, peas, or soybeans The proteins may be isolated from these seeds and fed with other pure chemical substances in synthetic diets or the meals themselves may be extracted with warm 70 per cent alcohol to remove choline and betaine and, combined with minerals vitamins fats and carbohydrates to produce hypolipotropic diets TABLES 1 and 2 show a few of the protein mixtures which we have used in our laboratory to produce hypolipotropic diets causing fatty livers, hemorrhagic kidneys or cirrhosis

To each of these protein mixtures the same amounts of minerals vitamins fats and carbohydrates were added to produce choline-free diets with essentially the same total protein content and the same total methionine content Yet Diet 2 proved as we anticipated to be more severely hypolipotropic than did Diet 1 The first diet, although it contains 20 per cent of protein is inadequate with respect to tryptophane threonine and phenylalanine Rats consuming Diet 1 barely grow at all The much better assortment of essent

TABLE 1

Diet 1		*Diet 2*	
Casein	8%	Casein	6%
Gelatin	12	Peanut meal (Alc ext d)	30
Total protein	20	Total protein	21
Total methionine	360 mg	Total meth onine (Copeland & Salmon[41])	360 mg

amino acids in Diet 2 permits good growth But when growth is improved the choline requirement is increased and, at the same time, the amount of dietary methionine available for lipotropic purposes is diminished Thus the second diet produces fatty livers of greater severity than does Diet 1 and produces them more rapidly The increased rate of production and greater severity of the lesions caused by Diet 2 is seen most dramatically in the development of hemorrhagic kidney lesions in weanling rats

TABLE 2 presents the composition of the protein moiety of several other diets that have been used in our laboratory when still more severely hypolipotropic conditions are desired

The last diet shown in TABLE 2 contains less than one third the quantity of methionine present in the widely used and severely hypolipotropic mixture of 30 per cent peanut meal (extracted with alcohol) and 6 per cent casein suggested by Copeland and Salmon[41] Diet 6 causes very severe signs of choline de [illegible]

[illegible]

been used by many workers, is not nearly as hypolipotropic as one containing 20 per cent arachin or as one containing 12 per cent arachin with 2 per cent fibrin or 8 per cent peanut meal plus 8 per cent of soybean protein Yet all [illegible]

many others were slow to appreciate

When one attempts to compare the curative properties of lipotropic agents and proteins in experimental or clinical cirrhosis, one really needs at least four groups of experimental animals The characteristic features of the four dietary regimens to be compared are shown in TABLE 3

A protein mixture for the first diet (A) might be 6 per cent alcohol extracted peanut meal plus 6 per cent soybean protein This contains 9 per cent of [illegible]

on 50 per cent of alcohol extracted peanut meal plus 5 per cent soybean protein (total protein 30 per cent, methionine 350 mg) To produce Diet D one might add 150 mgm DL methionine (making the total methionine about 500 mgm,

TABLE 2

Diet 3		Diet 5	
Arachin	12%	Arachin	12%
Gelatin	6	Fibrin	2
Casein	3	Casein	1
Fibrin	1		
Total protein	22 %	Total protein	15%
Total methionine	250 mg	Total methionine	150 mg
Diet 4		**Diet 6**	
'eanut meal (Alc ext'd)	12%	Peanut meal (Alc ext'd)	6%
)ybean protein	12	Soybean protein	6
tal protein	18 %	Total protein	9%
tal methionine	192 mg	Total methionine	96 mg

TABLE 3

A Low protein Hypolipotropic	*C* High protein Hypolipotropic
B Low protein Lipotropic	*D* High protein Lipotropic

as in Diet B) and 0.35 per cent choline chloride. Such a diet would ensure lipotropic adequacy. Omission of any one of the groups makes a valid comparison of the effect of protein *per se* with that of lipotropic agents, in the treatment of cirrhosis, impossible. No study of this type has yet been published.

Ideally, four more groups should be included, as shown in TABLE 4. These would permit us to separate out even more clearly the therapeutic factors of greatest influence. By low quality proteins, we mean in this connection those poor in methionine. A number of experiments along such lines were performed in our laboratory several years ago. Others are currently in progress and more are planned. In practically all of these we have tried to use diets in which all the essential amino acids were present in amounts sufficient to permit maintenance and repair of the tissues. We now look askance at curative experiments conducted with diets extremely low in protein since these obviously could not provide the building blocks needed for repair. Addition of protein to such a basal diet supplies the building blocks which choline alone cannot. Obviously when good protein, which contains methionine, is fed, both the building blocks and a source of choline are supplied simultaneously. From the therapeutic point of view this is ideal, but scientifically it is of no value in elucidating the fundamental mechanisms involved. Protein building blocks without choline or its precursors have always failed to give protection and, in cirrhosis caused by carbon tetrachloride, were shown to possess no curative value.[11]

TABLE 4

E	*G*
High protein (low quality) Extra methionine (DL)* No added Choline	Better quality protein No *free* methionine added No choline added
F	*H*
High protein (low quality) No extra methionine Choline chloride	Better quality protein Choline chloride

* An amount necessary to make the total methionine in Diets E and G equal

We attempt to resolve the first dilemma mentioned earlier by providing barely enough methionine to meet the requirements for growth and make the unsupported and, we believe, unwarranted assumption that none would be used for synthesis of choline This dilemma is further complicated by the well established increased need for lipotropes when the growth rate is augmented by adding methionine We should attempt to meet the more complicated second dilemma by using just enough methionine to eliminate the first dilemma and just sufficient vitamin B_{12} to satisfy other than lipotropic requirements, *i e*, trusting that none of either the vitamin B_{12} or methionine is "misappropriated" for lipotropic purposes The experimental evidence suggests that this ideal also may be impossible of attainment

and part of the second, would be avoided Failing this, we must try to find an otherwise innocuous chemical that blocks the enzymes transferring the methyl group of methionine to form choline Even more helpful would be the discovery of a substance that would selectively block methylation of aminoethyl alcohol This would resolve our present dilemmas and permit a breathing space until some new complications are revealed

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LIPOTROPIC EFFECTS OF VITAMIN B_{12} AND OTHER FACTORS

By Victor A. Drill
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Our interest in lipotropic substances other than methionine, casein, or choline followed the publication of studies of Gillman and Gillman on fatty livers in children with pellagra.[1] By means of serial biopsies of the liver, they were able to demonstrate a marked lipotropic of hog stomach (Ventriculin). They also reported a partial lipotropic effect of liver extract and a minimal effect of brewer's yeast.

A survey of the literature showed a lack of knowledge concerning the lipotropic effect of such supplements in experimentally induced liver injury. Hog stomach had not received any experimental study. With regard to liver extract, an early study by Rhoads and Miller[2] reported that liver extract only partially restored to normal the abnormal liver function produced in dogs by a black tongue diet. In 1942, György and Goldblatt[3] studied the effectiveness of a liver concentrate in preventing dietary cirrhosis in rats and concluded that the "liver extract was completely ineffective in preventing hepatic injury." The liver concentrate used in this later study was not commercial liver extract but the fraction of an aqueous liver extract soluble in alcohol. Yeast had received somewhat greater study but the results were conflicting.[3,4,5] Our first study, therefore, concerned the possible lipotropic effect of hog stomach, liver extract, and yeast.

The fatty livers were induced by a normal protein—high fat diet or a low protein—low fat diet (TABLE 1). Each rat also received a daily oral supply of the B vitamins as follows:

thiamin	20 μg
riboflavin	25 μg
pyridoxine	20 μg
calcium pantothenate	100 μg

Vitamins A and D were supplied by adding 5 drops of oleum percomorphum per kilo of diet.

In the first study, the following supplements were administered to rats receiving the high fat or the low protein diet: (a) crude liver extract (Lilly, 1 U.S.P. unit per cc.), 1 cc. subcutaneously 3 times a week, (b) brewer's yeast, 0.25 gm. per rat per day, mixed with the diet, (c) hog stomach (Ventriculin), 0.5 gm. per rat per day, mixed with the diet. Control groups of rats received the following: (a) choline chloride, 40 mgm. per day subcutaneously, (b) DL-methionine, 40 mgm. per day subcutaneously. One group receiving the high fat diet received 8 per cent additional casein in the diet, the content of corn starch being decreased by 8 per cent. Similarly, with the low protein diet, one group received an additional 0.5 per cent casein in the diet to control the small amount of protein added by the various supplements.

Untreated rats developed a marked fatty change, followed, depending on the length of the study, by fibrosis. The complete effectiveness of the choline

TABLE 1
EXPERIMENTAL DIETS

	Control diet %	Diet 1 %	Diet 2 %
ise n	16	16	6
rd	6	51	6
rnstarch	60	30	70
rose	15	—	15
t mixture	3	3	3
	100	100	100

TABLE 2
EFFECT OF VARIOUS SUPPLEMENTS ON FATTY LIVER

Group	No animals	Liver fat gm %
Exp 1 200 days (diet 16% protein 51% fat)		
High fat	13	20 5 ± 1 72
Ventriculin	10	21 7 ± 1 38
Methionine	10	17 8 ± 2 40
Casein 24%	9	15 4 ± 1 11
Yeast	10	11 2 ± 0 92
Liver extract	10	8 8 ± 0 65
Choline	10	7 3 ± 0 64
Exp 2 200 days (diet 6% protein 6% fat)		
High fat	4	23 1 ± 2 94
Ventriculin	4	21 4 ± 7 26
Methionine	4	17 8 ± 8 20
Yeast	4	7 8 ± 0 86
Liver extract	4	7 2 ± 0 56
Choline	3	7 2 ± 1 08

in preventing these lesions served as a base line to compare the lipotropic effects of the other supplements Microscopic examination or fat analyses indicated that the liver extract completely prevented the fatty changes in the liver of rats fed a high fat diet Supplements of Ventriculin or methionine were without effect casein was only slightly effective and yeast was partially effective (TABLE 2) Liver extract was equally potent in preventing fatty livers in animals fed the low protein diet With this diet the yeast supplement also offered complete protection whereas Ventriculin and methionine were without lipotropic activity [5, 6, 7]

Food intake was measured in each study and the lipotropic activity of the supplements was not related to changes in caloric or protein intake There was also no correlation between lipotropic activity and gain in body weight In further studies it was noted that oral liver extract 1 cc three times a week is slightly less lipotropic than the same dose given by injection [8]

The lipotropic activity of these supplements cannot be explained on the basis

TABLE 3
LIPOTROPIC EFFECT OF DIFFERENT VITAMIN B_{12} CONCENTRATES PREPARED FROM LIVER

No animals	Diet	Dose of conc in terms of µg of B_{12}	Liver fat gm %
Exp 1, 66 days			
4	Normal	—	5 6 ± 1 08
8	High fat	—	24 6 ± 0 59
10	High fat	1 0	7 8 ± 0 89
Exp 2 50 days			
6	Normal	—	6 3 ± 0 82
7	High fat	—	15 3 ± 2 01
5	High fat + #4	1 0	13 1 ± 1 01
5	High fat + #4	4 0	13 8 ± 1 27
5	High fat + #2	1 0	11 3 ± 1 35
5	High fat + #2	4 0	10 4 ± 0 95
5	High fat + #3	1 0	7 4 ± 0 88
5	High fat + #3	4 0	7 8 ± 0 62

of their choline content Of all of the supplements Ventriculin supplied the largest amount of choline namely, 1 8 to 2 1 mgm per day Although the liver extract completely prevented the fatty changes it supplied approximately [illegible]

for lipotropic effects

We then observed that certain vitamin B_{12} concentrates prepared from liver were also lipotropic[9] The activity of such extracts, however varies quite considerably[10] The extracts were injected three times a week in terms of their vitamin B_{12} activity and the total amount of extract administered was usually 0 5 cc three times a week The lipotropic potency of such extract can vary from inactivity partial effect to complete prevention of the fatty changes (TABLE 3) These vitamin B_{12} concentrates supplied only 0 2 mg of choline and 0 08 mg of methionine per day, an amount too small to explain their lipotropic activity One concentrate of vitamin B_{12} prepared from streptomyces broth was studied but proved to be too toxic to clearly evaluate lipotropic activity A growth promoting factor which has been highly purified [illegible][11, 12] This substance [illegible] ntrate seems to have [illegible] dy (TABLE 4) The higher dose of the extract however, was somewhat toxic[10]

In view of lipotropic effect of liver extract and vitamin B_{12} concentrate and the fact that their activity could not be explained on the basis of choline content further studies were designed using the high fat diet, in which small amounts of supplements were administered in different combinations to rats

TABLE 4

FFFECT OF VITAMIN B_{12} CONCENTRATE ON RATS FED A HIGH FAT DIET FOR 30 DAYS

Animals	Diet	B_{12} conc (3 × week)	Liver fat %
5	Low fat	none	7 02 ± 0 72
5	High fat	none	23 9 ± 1 40
5	High fat	0 3 cc	18 4 ± 0 24
3*	High fat	0 8 cc	11 9 ± 4 39

* Two animals died on the 12th and 26th days

TABLE 5

EFFECT OF SMALL DOSES OF SINGLE SUPPLEMENTS ON LIVER FAT

No animals per group	Normal diet	High fat diet untreated	High fat diet treated	Supplement (3 × week)	Dose	Days of exp
10	4 8 ± 1 20	17 5 ± 1 49	14 1 ± 1 77	B_{12}	1 μg	30
8–10	6 8 ± 0 31	28 8 ± 1 03	27 3 ± 1 12	B_{12}	2 5 μg *	64
8–10	8 4 ± 1 05	26 7 ± 1 83	26 2 ± 2 54	B_{12}	1 0 μg	30
			24 6 ± 0 83	B_{12}	4 0 μg	30
6	—	18 9 ± 1 98	18 3 ± 2 54	B_{12b}	1 0 μg	64
10	4 8 ± 1 20	17 5 ± 1 49	23 4 ± 1 83	Choline	1 0 mg	30
			26 1 ± 2 38	Choline	2 0 mg	30
6	—	18 9 ± 1 98	16 4 ± 1 27	Folic acid	2 5 mg	64
			21 0 ± 3 81	Folic acid	25 0 mg	64
			16 3 ± 2 19	Citrov fact	2 500 units	64
			16 8 ± 1 67	Citrov fact	25 000 units	64
6	6 3 ± 0 29	24 9 ± 1 14	20 7 ± 1 85	Inositol	2 0 mg	33
			18 9 ± 2 38	Inositol	4 0 mg	33
8	5 8 ± 0 27	19 2 ± 1 67	16 4 ± 1 72	Aureomycin	5 0 mg	31

* 6 times per week

receiving a high fat diet The amounts of supplements administered were similar to those present either in liver extract or vitamin B_{12} concentrate Although small amounts of each material present in liver extract may be ineffective by itself it may, when given in combination with other materials, demonstrate lipotropic activity The single supplements studied were B_{12}, B_{12b}, choline folic acid citrovorum factor, inositol, and aureomycin Each of these alone (TABLE 4) in the doses used were ineffective in preventing fatty changes in the liver of animals fed a high fat diet [8, 14, 15] Gyorgy and Rose also reported that crystalline vitamin B_{12} was without lipotropic effect in animals fed a high fat diet They did, however, obtain a partial lipotropic effect of

of renal hemorrhagic necrosis in rats Our attempts to demonstrate a sparing effect of B_{12} on choline in the lipotropic studies were unsuccessful (TABLE 5) The doses of choline were graded and were greater than the amount of choline present in liver extract or vitamin B_{12} concentrate [8] Thus the lipotropic of liver extract cannot be explained by a sparing effect of vitamin B_{12} on the amount of choline present A combination of choline and vitamin

TABLE 6

EFFECT OF CHOLINE, VITAMIN B_{12}, AND VITAMIN B_{12b} ON LIVER FAT OF RATS FED A HIGH FAT DIET

No animals	Diet	Choline mgm	B_{12} µg	B_{12b} µg	Liver fat gm %
		Experiment 1, 30 days			
10	Normal	—	—	—	4.8 ± 1.20
10	High fat	—	—	—	17.5 ± 1.49
9	High fat	0.5	1.0	—	19.0 ± 1.53
10	High fat	1.0	1.0	—	23.5 ± 1.26
10	High fat	2.0	1.0	—	18.9 ± 2.24
		Experiment 2, 64 days			
6	High fat	—	—	—	18.9 ± 1.98
5	High fat	2.0	—	1.0	20.1 ± 2.27
		Experiment 3, 72 days			
4	Normal	—	—	—	5.2 ± 0
5	High fat	—	—	—	23.7 ± 1
6	High fat	4	2	—	19.2 ± 0

TABLE 7

LIPOTROPIC EFFECT OF CHOLINE, VITAMIN B_{12} AND B_{12b} IN COMBINATION WITH FOLIC AND CITROVORUM FACTOR

No animals	Diet	Choline mg	B_{12} µg	B_{12b} µg	Folic acid µg	Citrov factor units	Liver fat gm %
			Experiment 1, 64 days				
6	High fat	—	—	—	—	—	18.9 ± 1
6	High fat	2	1	—	—	2,500	16.9 ± 1
6	High fat	2	1	—	—	25,000	11.4 ± 1
6	High fat	2	1	—	2.5	—	12.1 ± 0
6	High fat	2	1	—	25.0	—	11.4 ± 0
5	High fat	2	—	1	—	25,000	10.7 ± 1
5	*High fat*	*2*	—	*1*	*25.0*	—	*10.5 ± 1*
			Experiment 2, 72 days				
4	Normal	—	—	—	—	—	5.2 ± 0.75
5	High fat	—	—	—	—	—	23.7 ± 1.72
6	High fat	2	1	—	2.5	—	14.4 ± 1.12
7	High fat	4	2	—	2.5	—	14.3 ± 0.78

B_{12b} was also ineffective[14] and vitamin B_{12} does not exert a sparing effect on methionine in lipotropic studies[16] (TABLE 6).

During the past two years studies were also performed with folic acid or citrovorum factor in combination with choline and vitamin B_{12}. In the previous studies the liver extract supplied an average 1.2 µg and the vitamin B_{12} concentrate an average of 0.03 µg of folic acid per day. The addition of

TABLE 8

LIPOTROPIC EFFECT OF CHOLINE, VITAMIN B_{12}, FOLIC ACID AND INOSITOL

No. animals	Diet	Choline mg	B_{12} µg	Folic acid µg	Inositol mg	Liver fat gm %
			Experiment 1 92 Days			
12	Normal	—	—	—	—	6.8 ± 0.49
12	High fat	—	—	—	—	18.7 ± 1.39
9	High fat	0.5	—	1	0.5	20.1 ± 0.58
10	High fat	1.0	—	2	1.0	17.7 ± 2.48
			Experiment 2 33 Days			
6	Normal	—	—	—	—	6.3 ± 0.29
6	High fat	—	—	—	—	24.9 ± 1.14
6	High fat	2.0	—	2.5	2.0	19.5 ± 1.10
6	High fat	2.0	—	10.0	2.0	17.3 ± 3.25
6	High fat	2.0	1.0	2.5	2.0	14.9 ± 1.62
5	High fat	2.0	1.0	10.0	2.0	16.8 ± 2.30
6	High fat	2.0	1.0	10.0	4.0	15.8 ± 2.10

TABLE 9

LIPOTROPIC EFFECT OF SMALL AMOUNTS OF VARIOUS SUBSTANCES

No. animals	Diet	Choline mg	B_{12} µg	Folic acid µg	Inositol mg	Aureomycin mg	Liver fat gm %
6	Normal	—	—	—	—	—	5.8 ± 0.27
8	High fat	—	—	—	—	—	19.2 ± 1.67
8	High fat	2	—	10	4	5	17.6 ± 2.05
9	High fat	2	1	10	—	5	11.0 ± 0.83
8	High fat	2	1	10	4	5	12.6 ± 1.71

folic acid to the choline and the B_{12} produced a partial lipotropic effect.[14] This effect of folic acid could also be obtained by administering 25,000 units of citrovorum factor with the B_{12} and choline, and vitamin B_{12b} was also effective in replacing vitamin B_{12} in such a combination of supplements (TABLE 7). These three supplements were not as effective as liver extract, for in each study the liver fat was only returned about half way to normal.

In the earlier studies, the liver extract supplied 0.9 mg. of inositol per injec-

Discussion

The establishment in 1948 of the lipotropic activity of liver extract in rats fed a high fat diet demonstrated that factors other than choline can markedly influence the fat content of the liver.[8] The lipotropic effects of vitamin B_{12} concentrates prepared from liver are also not explainable on the basis of choline content.[9,10] That vitamin B_{12} was important was demonstrated by Schaefer

and co workers who reported a sparing action of this vitamin on the choline requirement of rats and chicks [17, 18] Vitamin B_{12} and other factors may act to alter either the rate of synthesis of labile methyl groups or the process of transmethylation, but the exact mechanism of such effects is not known It is now known that the methyl groups of choline and methionine can be synthesized

concluded that supplements of vitamin B_{12}, folacin, and homocystine did not prevent the renal injury and death of weanling rats (40–50 gm) in the absence of a dietary source of labile methyl groups [26] The variation in results may be due to the difference in requirement among the strains of rats [27, 28]

Other studies have also shown that vitamin B_{12} is necessary for the methylation of homocystine to form methionine A diet deficient in vitamin B_{12} interferes with the ability of the liver to convert betaine and homocystine to methionine or choline and homocystine to methionine [28] Jukes, Stokstad and Broquist observed that vitamin B_{12} was necessary for the methylation of homocystine to obtain growth in chicks fed a methionine deficient diet [30, 3] This action of vitamin B_{12} depends however, on the amount of methyl groups available When weanling rats are fed a diet deficient in choline and methionine it was observed that choline alone will prevent the renal injury but gain in body weight is very slight In the presence of choline Schaefer and Knowles[26] observed that supplements of vitamin B_{12} alone allowed an adequate gain in weight, indicating that the synthesis of methionine from homocystine was facilitated When, however, the amount of methyl groups was limited by a further reduction of choline intake both vitamin B_{12} and folacin were required to allow adequate growth

A deficiency of folic acid in the chicken has also been reported to interfere with the methylation of homocystine [31] and still other studies show the need for both folic acid and vitamin B_{12} Schaefer and Knowles[26] showed that supplementations of a basal diet with homocystine, betaine, and aminoethanol failed to support growth or prevent renal injury With 0 41 per cent betaine and 0 05 per cent aminoethanol in the diet the addition of vitamin B_{12} prevented renal injury and permitted good growth With a lesser amount of betain and aminoethanol in the diet supplements of both vitamin B_{12} and folic acid were required to prevent renal injury and allow growth Such weanling rats fed deficient diets also develop fatty livers The addition of vitamin B_{12} and folic acid suppressed the accumulation of fat in the liver of rats fed subprotective levels of choline [32]

Of particular interest is the relatio[illegible] acid to choline oxidase and betaine aldehyde o[illegible] hich are r[illegible] transmethylation reactions from choline in[illegible] been re[illegible] ious groups that amino pterin a folic [illegible] inhibits [illegible] activity [33, 34, 35] Wil liams[36] has show[illegible] rm n[illegible] ine oxidase activity but also [illegible] oxi[illegible] nd anaerobic transmethylation [illegible] It [illegible] of folic acid

plays a part in the conversion of choline or betaine aldehyde to the actual methyl donor, betaine, and its transmethylation to homocystine Stekol *et al* have found that a deficiency of folic acid in the adult rat reduces the capacity to utilize glycine or serine for the production of choline During vitamin B_{12} [illegible]

extract, the various substances present in liver extract were administered alone and in various combinations The amount of each supplement was equal to or slightly greater than that present in liver extract or vitamin B_{12} concentrates The small amounts of choline or vitamin B_{12} present in such liver extract were [illegible]

to enable a sparing effect of vitamin B_{12} to be demonstrated The further addition of folic acid to a combination of vitamin B_{12} and choline produced a partial reduction in liver fat The amount of folic acid used was about ten times that present in liver extract The folic acid was not effective alone and had to be used in combination with choline and vitamin B_{12} The further addition of inositol or aureomycin failed to influence further the deposition of fat in the liver The best results, therefore, have been obtained with a mixture of these three supplements and there must be other factors in the liver extract that can influence the synthesis of labile methyl groups

With regard to other diets and results, Gyorgy and co-workers reported that liver extract may enhance the effect of casein or of methionine in treating experimental cirrhosis[39] and that vitamin B_{12} does not affect the production of necrosis with a necrogenic diet [16] Using normal diets, Popper[40] has also found that vitamin B_{12} concentrate will inhibit the fatty changes and depletion of [illegible]

cessation of carbon tetrachloride, however, brought about a more rapid reversal of the hepatic damage Hove has also demonstrated that vitamin E or vitamin B_{12} can increase the survival, under certain dietary conditions, of rats receiving toxic doses of CCl_4 [42] The choline oxidase activity of the liver was not influenced by vitamin E or by CCl_4 [43]

Aureomycin may exert a sparing effect on vitamin B_{12}, as measured by growth response in rats and chickens,[44 45 46] and Gyorgy has reported that aureomycin can exert a lipotropic effect and an antinecrotic effect in experimental animals [47 48] It is generally assumed that aureomycin given orally acts to alter the bacterial floor of the gut This may occur by a decrease in the number of *E coli* in the gut and a simultaneous increase in *Bacillus megatherium* an organism that can produce large quantities of vitamin B_{12} [49] In our own studies, the administration of aureomycin by injection did not improve the partial lipotropic effect of folic acid, vitamin B_{12}, and choline

The problem becomes more complicated by interrelationship between cortisone, vitamin B_{12}, and folic acid Cortisone will increase the excretion of vitamin B_{12} in pigs fed a vitamin B_{12} deficient diet and thereby reduce body growth and survival[50] Vitamin B_{12} or aureomycin can largely counteract the growth inhibiting effects of cortisone in rats[51] Cortisone can also replace pteroylglutamic acid and citrovorum factor for the growth of *Streptococcus faecalis* and *Leuconostoc citrovorum* and can increase the growth inhibiting effects of aminopterin on *Leuconostoc citrovorum*[52]

Summary

The lipotropic effect of liver extract or certain vitamin B_{12} concentrates prepared from liver cannot be accounted for by their content of choline Using various supplements in the amounts present in these extracts it was found that a combination of choline, vitamin B_{12}, and folic acid produced a partial lowering of liver fat in rats fed a high fat diet In the absence of any one supplement from this mixture liver fat was not even partially reduced Vitamin B_{12} could be replaced by vitamin B_{12b} and citrovorum factor could replace folic acid The further addition of inositol and aureomycin was without added effect in lowering liver fat

Investigators have shown a certain relationship of vitamin B_{12} and folic acid to the synthesis of labile methyl groups and to their use in the transmethylation procedures as judged by the growth of animals or the prevention of renal injury It is evident that when diets are primarily designed to produce fatty changes in the liver, a factor or factors other than choline, vitamin B_{12}, and folic acid are required to allow maintenance of a normal liver fat

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Discussion of the Paper

trat on and purification of Factor 3 from natural sources especially from enzymatic casein digests we have found that certain fractions do *not* protect against dietary necrotic liver degeneration but actually *enhance* development of the disease We have accumulated much evidence that sources of methyl groups such as choline and betaine and also substances which are catalytically involved in one carbon unit metabolism such as vitamin B_{12} and citrovorum factor have such an enhancing effect on the development of liver necrosis (Schwarz Introduction page 617) According to our conception that there is an antagonistic balance between liver necrosis on the one hand and fatty liver and cirrhosis on the other hand we have tested our liver necrosis-accelerating fractions for protective activity against fatty infiltration of the liver In pre

dentified constituents the exact nature of which awaits further elucidation

LIVER CARCINOMA AND RELATED LESIONS IN CHRONIC CHOLINE DEFICIENCY*

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In studies of the pathology of chronic choline deficiency in the rat, the development of various types of neoplasms was observed in a significant percentage of experimental animals. Some of these results have been reported by Copeland and Salmon in 1946,[1] Engel, Copeland, and Salmon in 1947,[2] and Schaefer, Copeland, Salmon, and Hale in 1950.[3] Over 500 chronic choline deficient rats have been studied in this laboratory. Since the finding of neoplasms in these animals was most frequent in the liver, it is the purpose of this paper to describe the sequence of lesions culminating in the appearance of liver carcinoma. In another series of experiments, chickens were used to determine whether it would be possible to produce neoplasms by chronic choline deficiency in a species of animal other than the rat. The fatty changes, cirrhosis, and neoplasms of the liver, as well as some kidney changes which developed, are compared to corresponding lesions in the rat.

The general procedure followed and typical choline-deficient diets used in our laboratory have been described in previous publications.[1-4]

Histologic Studies on Livers of Chronic Choline Deficient Rats

Fatty livers and cirrhosis. The livers of rats that died during the first four weeks of the experiments were swollen and quite light in color. Microscopically there was a marked accumulation of fat uniformly involving all lobes of the liver, and apparently all the liver cells. On the other hand, sections from the livers of rats that had been on the choline deficient diet for as long as three months showed a zonal distribution of fatty infiltration with marked accumulations of fat in some areas forming peculiar architectural alterations.[1] These are similar to lesions described as "fatty cysts" by Hartroft and Ridout.[5] In later stages of the deficiency, the amount of fat appeared to decrease as cirrhosis advanced in extent and severity. Some lobules of the liver contained considerable fat but others seemed to be composed largely of newly regenerated liver cells. Ceroid was present in all cirrhotic livers but varied in amount. Mitotic figures were found in the cirrhotic livers indicating that regeneration of new parenchymal cells was taking place at a rapid rate. There was a pronounced variation in the size of the liver cells. Bile duct proliferation was seen in a majority of the livers.

Neoplasms of liver. In the livers of all the rats that remained on the choline deficient diets for eight months or longer, severe nodular cirrhosis was observed. Large regeneration nodules 0.5 to 1 cm in diameter were common. Tumors

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developed in a significant number of cases. The incidence of tumors of the liver in the rat varied in the different experiments from 16 1 to 30 per cent with an average of 23 1 per cent The tumors varied in size from 0 8 to 3.5 cm in diameter and were of a different consistency from the regeneration nodules The [illegible] appearance of one of the liver tumors is illustrated in FIGURE 1

2, 3, and 4

variation in

tumor The

alveolar arrangement and general appearance of the cells in some areas seem to point to a bile duct origin, but in most cases the cell type suggests an origin from liver cord cells

Histologic studies are being continued on all tumors that developed in chronic choline deficient rats in an attempt to determine which of these tumors are definitely malignant Special stains were carried out on most malignant or "possibly malignant" lesions Histologic determination of the malignancy of many of these "tumors" is a matter of judgment We have attempted to be conservative by grouping many of them under the classification "atypical regeneration nodules"

In many tumor cases, invasion of the mesentery was noted grossly and verified microscopically In some of these cases, little or no connection was noted

prove that these structures were blood vessels However, this did not eliminate the possibility of these structures representing lymphatic invasion The appearance of the tumor nodules in these areas suggests that, as the tumors spread into mesenteric tissue, they incite the production of a limiting fibrous wall One slide in each of two cases did show definite vein invasion With the exception of these two cases, the stroma of the tumor blended too well with the fibrous wall for that wall to be a blood vessel There were proliferating bile ducts at the edge of some of the tumor islands

An interesting feature in the study of periodic acid stains on sections of the

a large amount of collagen in the cirrhotic, nontumorous portions of the liver, but in the tumor proper, there was a relatively small amount around alveolar structures In slides prepared by Foot's modification of Bielschowsky s reticulum stain, there is a characteristic alveolar pattern in some parts of the tumor which is typical of epithelial origin

No fibrils arising from tumor cells were demonstrated by phosphotungstic acid stain There was some PAS positive material in some of the macrophages in the cirrhotic livers which is probably ceroid

All the blocks of lung tissue from choline deficient rats with liver tumors are

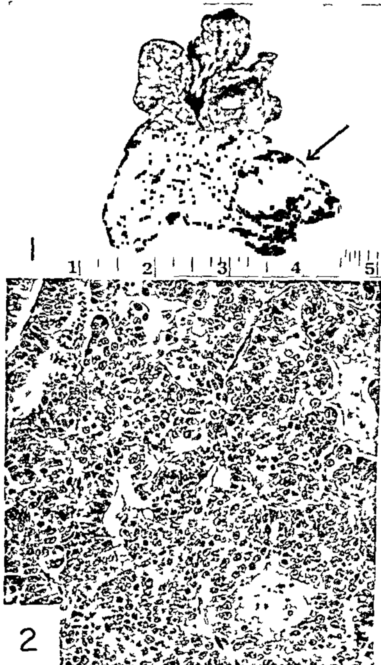
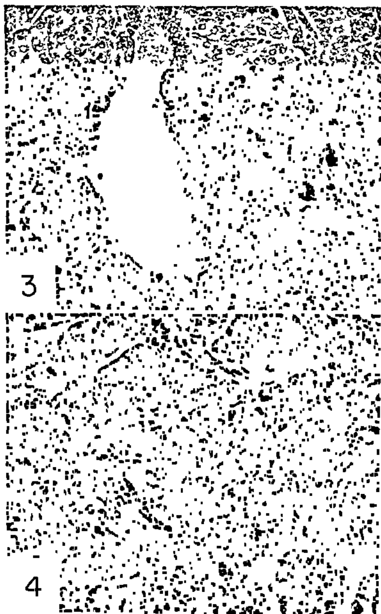


FIGURE 1. Photograph showing the typical gross appearance of a liver tumor from a choline-deficient rat. Reduced to three fourths actual size.

FIGURE 2. Photomicrograph of a section of the liver tumor illustrated in FIGURE 1. In this area the tumor cells form ductlike structures. Hematoxylin and eosin stain. X230.



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being recut In the process of this study, two cases of pulmonary metastases from liver tumors have been found In each case, an embolus was found in a blood vessel in the lung in addition to the metastatic nodule of tumor tissue A photomicrograph of an embolus of tumor tissue (from liver tumor) in a blood vessel in the lung of a choline deficient rat (no 14065) is shown in FIGURE 5 The pulmonary metastasis from this liver tumor is illustrated in FIGURE 6 A pulmonary blood vessel containing an embolus of tumor cells (rat no 14067) is illustrated in FIGURE 7 The pulmonary metastasis from a liver tumor in this rat is shown in FIGURE 8

Although only two cases of pulmonary metastases from liver tumors have been found to date in our choline deficient rats, this serves to prove that at least some of these tumors are malignant The microscopic appearance of the two metastasizing tumors did not differ materially from many of those in which no metastases were found

Histopathology of the Livers of Chronic Choline Deficient Chickens

Microscopic studies of slides prepared from the tissues of 105 chronic choline-deficient chickens and 33 control chickens fed the same diets supplemented with 0 3 to 0 6 per cent choline chloride have been made The chickens remained on the choline deficient diet 28 to 133 weeks (average 78 weeks) The control chickens remained on the diet for 44 to 133 weeks (average of 80 weeks) Of the 105 chickens, 38 developed neoplasms of various kinds which were multiple in 12 chickens No neoplasm was found in the 33 control chickens

Fatty liver and cirrhosis There was considerable variation in the gross appearance of the livers of the chronic choline deficient chickens The livers varied in size, the majority of them being very large and fatty An interesting feature of the fatty livers was that the entire liver was usually not involved Scattered areas on each lobe frequently were of a normal, dark red color, the remainder of the liver having a light colored, fatty appearance This was confirmed by microscopic studies of slides which showed some areas of normal liver tissue immediately adjacent to but sharply demarcated from, extremely fatty areas (FIGURE 9) The normal areas may represent regenerated liver tissue although they had the appearance of unchanged hepatic parenchyma Gross nodular cirrhosis so frequently found in the livers of choline deficient rats was observed in only two cases, but frequently was observed microscopically (FIGURE 10)

Neoplasms of liver Lesions resembling tumors were found in 23 cases Eleven of these were classified as atypical regeneration nodules, 10 as cholangiocarcinoma and 2 as hemangioendothelioma The gross appearance of one of the liver tumors is illustrated in FIGURE 11 The tumors varied in size from 0 8 to 3 cm in diameter The microscopic appearance of the liver tumors is illustrated in FIGURES 12, 13, and 14 There was a difference in the arrangement of the cells in different parts of the tumors varying from closely packed cells without any apparent order to well formed glandular and ductal structures In some cases the neoplastic cells closely resembled liver cells, but in most cases they were anaplastic FIGURE 12 illustrates one of the tumors in which

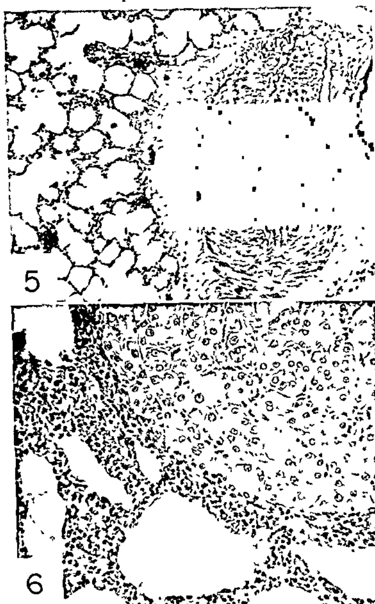


FIGURE 5 Photomicrograph of a blood vessel in the lung of choline-deficient rat no 140?5 containing an embolus of tumor cells from a hepatocellular carcinoma of the liver Hematoxylin and eosin ×150

FIGURE 6 Photomicrograph of a pulmonary metastasis from a liver tumor in choline-deficient rat no 14065 Hematoxylin and eosin ×310

FIGURE 7. Photomicrograph of an embolus of tumor cells in a blood vessel in the lung of choline-deficient rat no. 14067. Invasion from liver tumor. Hematoxylin and eosin. × 275.

FIGURE 8. Photomicrograph of a pulmonary metastasis from a liver tumor in choline-deficient rat no. 14067. Hematoxylin and eosin. × 10.

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FIGURE 9 Photomicrograph of a section of liver from chicken 713 to illustrate extremely fatty conditions surrounding areas in which the parenchyma is normal This may represent regenerated liver tissue Hematoxylin and eosin ×240

FIGURE 10 Photomicrograph of liver from chicken 211 This illustrates one of the cirrhotic areas which were found in the livers of chronic choline deficient chickens Hematoxylin and eosin ×450



11

12



the cells are so compactly arranged that cytoplasmic borders are not visi
The cytoplasm of the tumor cells does not stain as dark as the adjacent l
cells The nuclei have irregular shapes and contain one or two nucleoli
all the tumors, there were areas in which the cells were arranged in alve
Periodic acid stain reve
in less glycogen than the
like structures contained c
siderable amounts of pink staining material, probably mucus, thus being c
patible with ductal origin of the tumor Mucicarmine stains demonstra
only a small amount of mucus Mucus was not demonstrable in atypical
generation nodules or carcinomas with closely packed cells In slides prepa
by Foot's modification of Bielschowsky's reticulum stain, there was a he
reticulum network throughout these tumors with a characteristic alve
pattern The reticulum fibers occasionally extended between individual c
in the epithelial island, but characteristically did not No fibroglia w
demonstrable in the sections of liver tumors stained with phosphotungstic a
hematoxylin

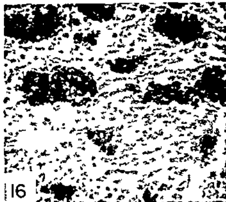
Increase in Iron Positive Pigment in Liver and Kidney of Chronic Choline Deficient Chickens

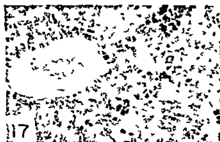
In studies of the microscopic appearance of tissues of chronic choline defici
chickens, an excessive deposition of pigment was observed in the livers a
kidneys Large accumulations of intracellular and extracellular pigment w
observed in the livers, but in the kidneys the pigment was mostly intracellu
The intracellular hepatic pigment was occasionally seen in histiocytes but
marily in epithelial cells In sections stained with hematoxylin and eosin,
pigment was found in the form of yellowish brown granules varying sligh
in size and depth of color and occasionally appearing as conglomerate mas

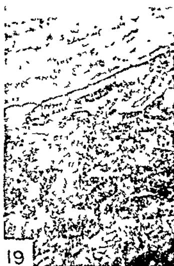
Iron stains The pigment gave a positive reaction for iron (Turnbu
blue) A photograph of an entire section of liver illustrating the positive i
reaction is shown in FIGURE 15 It is of interest to note that the atypical
generation nodule in the center of this section did not contain pigment, l
there was a large amount distributed throughout the rest of the liver
similar distribution was observed in the case of the livers that contained tum
A slide of liver containing a large amount of intracellular and extracellular p
ment is shown in FIGURE 16 The pigment in the epithelial cells is in the fo
of fine granules, but the extracellular deposits are large and small aggrega
of granules The general distribution of this pigment is illustrated in FIGU
17 A photomicrograph illustrating the deposition of iron positive pigment
the convoluted tubules of the kidney is shown in FIGURE 18 A slide illustr
ing the difference in the amount of pigment in the convoluted tubules a
collecting ducts of the kidney is shown in FIGURE 19

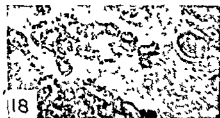
The pigment gave the positive stain reactions for iron and was, therefo











bility was further eliminated by the fact that tissues fixed by three different fixatives gave the same reaction

Discussion

The manifestations of choline deficiency in the chicken were somewhat different from those of the rat under the conditions of our experiments In contrast

to the uniform accumulations of fat in the livers of rats in early stages the chicken livers were not uniformly fatty In some cases entire lobes of a chicken liver contained very little fat even microscopically This feature may account for the fact that nodular cirrhosis was found to involve the entire liver in only two cases and that in the majority of cases only scattered areas of the livers were cirrhotic.

A definite hemochromatosis (defined as hepatic cirrhosis with iron deposits in the liver and other organs) developed in approximately 25 per cent of the choline deficient chickens while more than normal quantities of iron positive pigment were found in the livers and kidneys of nearly all the birds Slides of the liver and kidney from control chickens fed the same diet but with choline added were carefully checked for the presence of the pigment There was a small amount present in some of the older chickens The amount of pigment in the controls was extremely small in comparison to the large amount in the deficient chickens In contrast to the excessive pigmentation of the livers and kidneys of chronic choline deficient chickens the livers and kidneys of choline deficient rats showed only moderate amounts of the pigments The livers and kidneys of the choline deficient rats however did show a definite increase in iron positive pigments over the livers and kidneys of the control rats Only minute traces of the pigment could be seen in livers and kidneys of the control rats

Warren and Drake[7] have discussed the relationship between hemochromatosis and primary carcinoma of the liver in man and have published a pertinent bibliography Gillman, Mandelstam, and Gillman[8] have described the presence of large amounts of iron pigment in human cirrhotic livers in Africans whose diets appeared to be inadequate in quality

It should be pointed out that the diets of the rats and chickens were free of vitamin B_{12} and that the rat diets were also deficient in folic acid It has been shown in this laboratory that vitamin B_{12} has a definite choline sparing action[9, 10, 11] We have not found tumors in the livers of rats receiving choline deficient diets supplemented with vitamin B_{12}

Summary

(1) Studies have been carried out on chronic choline deficient rats and chickens The most prominent lesions have developed in the livers and kidneys of these animals

(2) In the rat liver fatty infiltration has developed consistently and has been followed eventually in a large number of cases by cirrhosis and in some cases by tumor formation Pulmonary metastases from liver tumors were found in two rats

(3) In the chicken liver focal rather than generalized reactions were usually observed Fatty changes and cirrhosis were common Lesions considered to be hepatic malignancies developed in 12 cases with 11 others showing atypical regeneration nodules

(4) Increased pigmentation with an iron positive pigment (hemosiderin) was observed in the livers and spleens of the choline-deficient rats and chickens but was much more severe in the chickens

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HEPATOMAS PRODUCED IN MICE BY FEEDING BENTONITE IN THE DIET

By J Walter Wilson

Department of Biology Brown University Providence, R I

It is the purpose of this paper to report the occurrence of hepatomas in mice fed a complete semisynthetic diet mixed with an equal amount of bentonite by weight The details of the experiments and the evidence that has led to the tentative conclusion that the hepatomas are the result, in part, of an induced choline deficiency are to be reported in detail elsewhere [1, 2] Bentonite is a [illegible] s of [illegible] ing

Mice can adapt themselves to eating a diet containing half its weight of such inert material as Ruffex or barium sulphate with only a slight decrease in growth rate (FIGURE 1) Where bentonite makes up 25 per cent of the diet growth is also nearly normal, but with 50 per cent bentonite in the diet growth is almost completely suppressed (FIGURE 2) The mice continue to live however, and grow very gradually for several months, but do not reach a normal size Since the mice eat as much food on the 50/50 Bentonite diet as on the *50/50 Ruffex diet, failure to grow is not due to inanition alone*

Histological studies reveal that after the first three weeks there is more or less fat in the livers (PLATE 1, 1), apparently depending on whether or not the mouse is gaining weight This is in keeping with the general observation that even on a diet free of lipotropic substances the liver may not contain fat if the animal is not growing The fat appears first as fine droplets but soon many of the liver cells are distended with single large drops Eventually after three or four months there is some fibrosis with progressive loss of architecture (PLATE 1, 2), although typical 'hob nail' cirrhosis has not been seen There is also the accumulation of masses containing a brownish pigment, apparently ceroid (PLATE 1, 3)

Hepatomas have been found in all but one of twelve mice that in three experiments have been kept over 200 days on the diet These are typical benign hepatomas of mice much like those produced by repeated CCl_4 treatment or occurring spontaneously in C_3H mice

The fatty livers fibrosis ceroid and hepatomas present a picture much like that we have previously reported in mice maintained for several months on a diet low in protein and deficient in choline [3] The symptoms develop more gradually and the mice maintain a more thrifty appearance On a choline-deficient diet, at sporadic intervals individual mice suddenly become anemic and die, so that only a few have been kept on it long enough to develop hepatomas It seems possible that in these sudden deaths, toxic materials produced by the intestinal flora may be involved If so, they may be removed by the bentonite which might account for the more thrifty condition of animals on the 50/50 bentonite diet

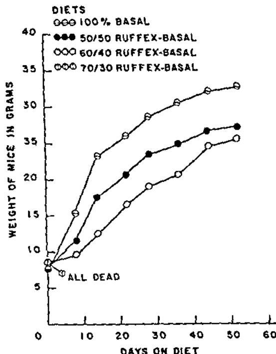


FIGURE 1

We have found that added choline will prevent the development of the fatty livers on the 50/50 bentonite diet, but that normal growth is only partially restored (FIGURE 2) When the amount of casein is doubled at the expense of corn starch, growth is also partially restored and the livers do not become fatty (FIGURE 3) This would be true of choline deficiency in both cases When 1 per cent dl methionine is added to the 50/50 bentonite basal diet, however, the mice develop fatty livers and fail to grow (FIGURE 4) As a tentative explanation of this anomalous result, we suggest that the bentonite removes dibasic amino acids, and that the added methionine precipitates an incipient [illegible] that described for rats deficient in essential amino acids, which we have not as yet been able to cure

It seems then that the deficiency is not a simple choline deficiency but may involve other essential basic materials removed from the intestinal contents by bentonite It would be expected that some of the larger basic molecules would be taken up and held more firmly than inorganic cations, for with

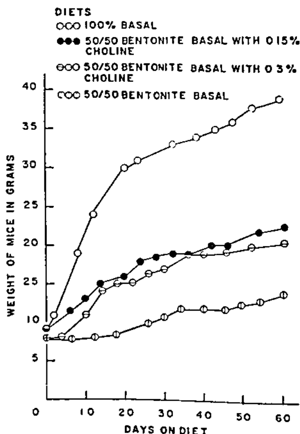


FIGURE 2

them, in addition to the Coulo rces Van der Waals f into play and bases so held are only ulty eluted[4] It w ifficult to predict what would be tak uch complicated s occur in the passage of the digestive riments designed us possi bilities are under way

Except for the appearanc are no strikin es for the first four weeks By ere is a pron size of particularly bular area cells a enlarged a homogen and he basophi icuous in region there a cuoles animal e added re n any mo ived tl gro the enla n con i Similar l nu

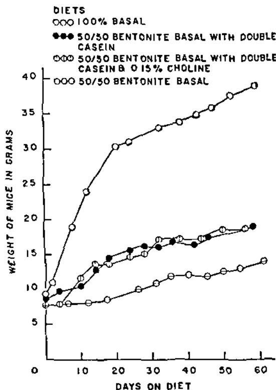


FIGURE 3

mice when they have been fed a diet containing a carcinogenic azo dye for a few weeks[5]

Th

enlarged liver cells are placed on a normal diet, rapid growth resumes and a spectacular wave of mitosis occurs in the liver which involves the large cells as well as the small ones (PLATE 2, 3) The large nuclei are obviously polyploid for they have very wide angled spindles and very large numbers of chromosomes The metaphase illustrated in PLATE 2, 3 has a few chromosomes that have failed to reach the equatorial plate From their size the numbers on the plate must be great T

Many of the figures are
anaphase and telephase (PL

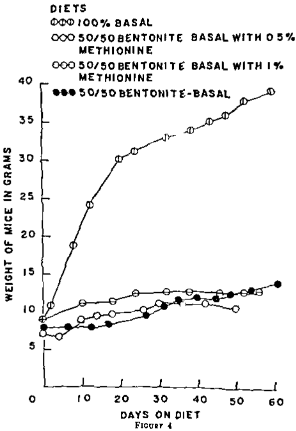


FIGURE 4

this sort in other cases where mi also when the liver, regenerating Whether they result in viable cells and thereby play a role in carcino speculation, and is worthy of furt observation of Hauschka and Le some num ascites tumors of

the mouse liver[6] hepatectomy, chromosome suggests[7] is particularly ice of abn

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4 HENP cations Chem exchange n adsorp

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7 BRUES A M & L REITZ 1951 Effects of external and nternal ra lat on on cell div sion Ann N Y Acad Sci 51 1497

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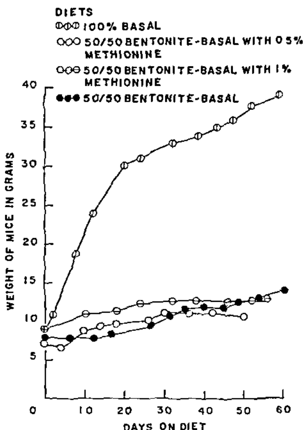


FIGURE 4

this sort in other cases where mitosis occurs in the mouse liver[6] They occu also when the liver, regenerating after partial hepatectomy, is irradiated Whether they result in viable cells with abnormal chromosome complemen and thereby play a role in carcinogenesis as Brues suggests,[7] is an interestin speculation, and is worthy of further investigation particularly in light of th observation of Hauschka and Levan[8] on the occurrence of abnormal chromo some numbers in ascites tumors of mice

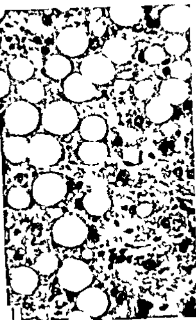
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Plate 1

1 Fatty liver of mouse on 50 50 bentonite basal diet with 1 per cent methionine for 49 days *Ocul*



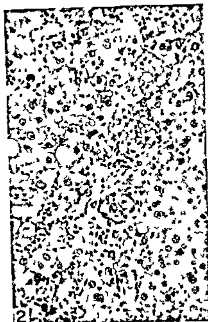
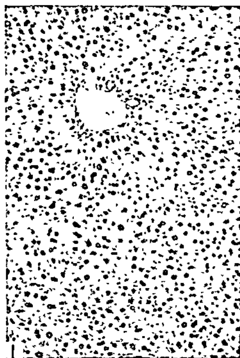






PLATE 1



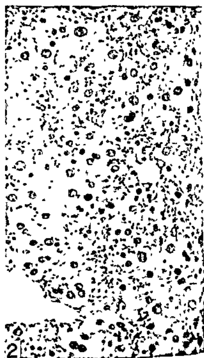
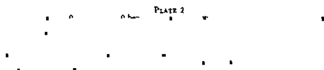






PLATE 2


Plate 2

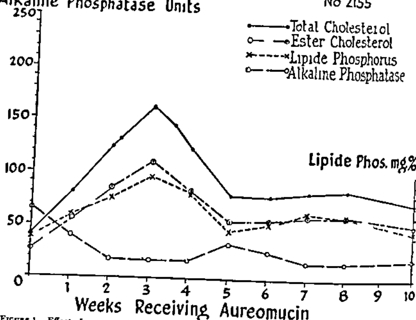


FIGURE 1 Effect of aureomycin *per os* upon the serum lipids and alkaline phosphatase activity of pancreatic duct ligated dog no 2155

regression took place and the concentrations of serum lipids leveled off at approximately 50 per cent of the peak values These were still well above the control concentrations The serum alkaline phosphatase activity rose somewhat during this period but did not approach the initial value

Dog No 2661 The pancreatic ducts of this dog were ligated 44 weeks prior to the antibiotic experiment Like the preceding animal this dog responded to aureom[illegible]

due [illegible] 5

serur[illegible] 1

[illegible] The maximum changes were reached in four weeks but were not as marked as in the previous animal, corresponding more closely to the plateau values These alterations, however, were sustained for an additional 10 weeks at which time aureomycin was withdrawn from the diet There was an [illegible]

alkaline phosphat[illegible] 1

Dog No 1921

previous to this e[illegible] showed the customary low blood lipids and high serum alkaline phosphatase activity (FIGURE 3) Three weeks after the administration of aureomycin the serum cholesterol [illegible]

[illegible] the control and peak levels At this time the animal was given daily 6 mcg of vitamin B_{12} subcutaneously and 5 mgm. of

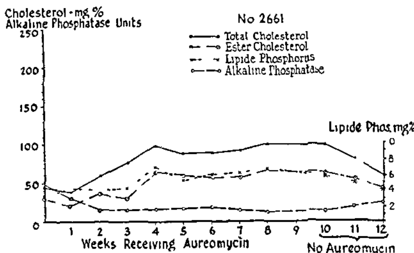


FIGURE 2 Effect of aureomycin *per os* upon the serum lipids and alkaline phosphatase activity of pancreatic duct ligated dog no 2661

folic acid orally in addition to the aureomycin The serum cholesterol and lipid phosphorus promptly rose to peak levels and were maintained at this point for three weeks Alkaline phosphatase activity also fell to its lowest level Upon withdrawal of the aureomycin from the diet while continuing the above vitamin administration, the serum lipids fell and the alkaline phosphatase activity rose These results indicate that the lipotropic action of aureomycin exceeds that of the vitamin B_{12} and folic acid It should also be pointed out that, one year previously, no effect on serum lipids or alkaline phosphatase activity was obtained in dog 1921 or in one other animal by the oral administration of 25 mcg of vitamin B_{12} plus 5 mgm of folic acid, nor by the subcutaneous administration of 100 mcg of vitamin B_{12} alone, or 6 mcg of vitamin B_{12} plus 4 5 mgm of folic acid No antibiotics were given at that time

In searching for the mechanism by which aureomycin was exerting its lipotropic effect, consideration was given to the possibility that, through its antibacterial action, less methyl acceptors may be formed Although little is [illegible]

excretion of ergothioneine The possibility of other methyl acceptors being involved in this condition cannot be eliminated

In view of the reports by Huerga and Popper[28, 29] that the major portion of a test meal of choline is converted to trimethylamine and its oxide by intestinal bacteria and that this conversion can be temporarily inhibited by pretreatment

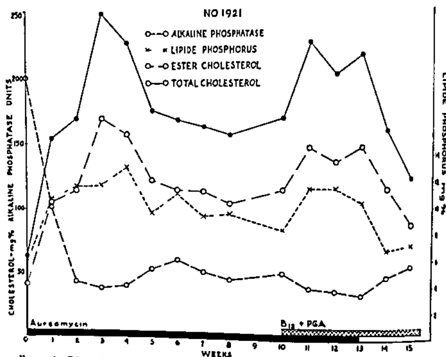


FIGURE 3 Effect of aureomycin alone and in conjunct on with vitamin B_{12} plus folic acid upon the serum lipids and alkaline phosphatase activity of pancreatic duct ligated dog no 1921

TABLE 1
THE EFFECT OF AUREOMYCIN INGESTION UPON THE EXCRETION OF ERGOTHIONEINE BY DOGS WITH LIGATED PANCREATIC DUCTS

Weeks receiving aureomycin*	Weekly excretion of ergothioneine (mg)		
	Dog 2155	Dog 2661	Dog 1921
none	862	810	725
1	1254	598	978
2	900	1078	888
3	1652	1444	655
5	1011	1373	
6			922

* 1 0 g daily for the first 3 weeks and 0 75 g daily for the remainder

with antibiotics, some measurements* of total trimethylamine excretion w made in some of our treated animals No consistent depression of chol destruction was found during prolonged aureomycin therapy If less chol were being converted to trimethylamine or its oxide, the action was tempora and of insufficient magnitude to explain the lipotropic effect of the antibiot

Discussion

These limited data indicate that aureomycin does have a lipotropic acti when given *per os* to dogs with ligated pancreatic ducts, as shown by a mark

* We are indebted to Dr J de la Huerga of Hektoen Institute Chicago Illinois for these analyses.

rise in serum cholesterol and phospholipid levels and a dramatic fall in serum alkaline phosphatase activity. Conclusive demonstration of a lipotropic effect, however, depends upon liver biopsy studies. Such experiments are under way.

The inability of aureomycin to maintain a normal level of serum cholesterol and phospholipids as do lipotropic substances such as choline or methionine, may be explained by partial bacterial adaptation to the antibiotic. In view, however, of recent reports[20, 21] that the administration of aureomycin or terramycin may cause a fine, fatty infiltration of the liver, this latter possibility has to be taken into consideration. Nevertheless, it appears from our limited data* that fatty infiltration of the liver induced in the dog by the antibiotic *per se* is minimal if present at all, but more animals are needed to settle the question. This is in agreement with the finding of Lepper *et al*[22] that a very large oral dose of antibiotic is required to produce fatty liver changes in mice.

Gyorgy and his associates were the first to demonstrate that aureomycin and other antibiotics exert a lipotropic action when fed to rats receiving a low protein, low choline, high fat diet[3] and that the same antibiotics delay the onset of liver necrosis when rats are fed a necrogenic diet.[20, 21] The recent [illegible]

in rats suffering from a choline deficiency or in dogs with a deficiency of pancreatic exocrine secretions is associated with its antimicrobial action. This [illegible]

tion of choline and methionine by the bacteria themselves.

Schaefer *et al*[24] and Burns and McKibben[25] have reported a lipotropic effect [illegible]

aureomycin, however, at a time when the lipotropic effect of the latter had [illegible]

under the same conditions. This suggests that the degree of choline deficiency or the bacterial flora of the intestine may be factors influencing the lipotropic effect of vitamin B_{12} and folic acid.

Summary

(1) The oral administration of aureomycin to dogs with ligated pancreatic ducts has a lipotropic effect as manifested by a marked rise in the existing low concentrations of serum cholesterol and lipid phosphorus to values approaching normal. The elevated serum alkaline phosphatase activity falls sharply

* A normal dog was given by mouth 1.0 gm aureomycin daily for 3 weeks and 0.75 gm daily for an additional 2 weeks before it was sacrificed. Total liver lipids were at the upper limit of normal, namely 4.9 per cent.

(2) These lipid and enzyme changes reach their maximum in three weeks and then recede and level off at some intermediate value

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SURVEY OF THE WORLD SITUATION ON KWASHIORKOR

By J F Brock

Department of Medicine, University of Cape Town Cape Town, Union of South Africa

A world survey is of value in this scientific monograph because the study of the ecology and epidemiology of kwashiorkor* may provide useful clues to the etiology and pathogenesis of cirrhosis and primary carcinoma of the human liver

The term "kwashiorkor" now has, for better or for worse, the sanction of the Joint F A O/W H O Expert Committee on Nutrition, which has quoted and presumably endorsed the view expressed by Brock and Autret that a wide and general appraisal of its nature and relationships suggests that it is the most serious and widespread nutritional disorder known to medical and nutritional science" In the African continent, the term has been accepted almost unanimously as replacing "malignant malnutrition," "infantile pellagra," "diboba," "mbuaki," and other local names

No attempt will be made in this communication to trace the history and details of this syndrome and its bibliography except in so far as they are relevant to the title of this monograph Brock and Autret[5] have done this and have made acknowledgment to the pioneers in this field in Africa They defined kwashiorkor as seen in the African continent in the following terms 'It is recognized that the syndrome cannot at present be accurately defined and that it merges into other nutritional syndromes, such as marasmus and Mehlnahrschaden, but it is felt that its prevalence and importance justify a tentative definition as follows

"A nutritional syndrome (or syndromes) found among indigenous Africans in which characteristically there occurs

"(a) retarded growth in the late breast feeding, weaning, and post weaning ages, with

"(b) alterations in skin and hair pigmentation,

"(c) oedema

"(d) fatty infiltration, cellular necrosis, or fibrosis of the liver,

"(e) a heavy mortality in the absence of proper dietary treatment, and

"(f) the frequent association of a variety of dermatoses

"Some combination of the above features appears to be fundamental other clinical features are so frequent as to be dominant if not fundamental"

The African aspects of kwashiorkor were brought up to date by the Second Conference on Nutrition called by the Commission for Technical Cooperation in Africa South of the Sahara (C C T A) in November 1952 at Fajara Gambia West Africa[7] The Joint F A O/W H O Expert Committee on Nutrition[19] held its third session at Fajara immediately after the C C T A Conference The members of the Committee had the advantage of hearing

* -raph is / called / in V on / tion of

the full discussion at the C C T A Conference and subsequently considered kwashiorkor in relation to the problems of nutrition in mother and child throughout the world

In the light of these two meetings, the definition and description given by Brock and Autret can be modified or expanded in certain respects The urative effect of reconstituted skim milk powder has been amply confirmed nd this might be listed as a fundamental and often critical diagnostic feature s predicted by Brock and Autret, vegetable protein has proved to be capable substitution to a limited extent for milk protein [10] We can conclude, erefore, that the deficient factor or factors are among the constituents of ried skimmed milk Whether the deficiency pattern is (1) a simple disproportion between total protein and calories, or (2) an amino-acid imbalance, or (3) a deficiency of accessory food factors yet to be identified, remains an open question The Joint F A O/W H O Expert Committee on Nutrition has very properly left this matter open in its tentative definition of "protein malnutrition"

There is no further confirmation of the claim by Auffret and Tanguy[1] that the milk of African mothers is deficient in methionine Bigwood[3] has pressed the importance of cystine deficiency Trowell, Davies, and Dean"[9] have brought their description of kwashiorkor in Uganda up to date Davies[8] has given an account of the pathology of the syndrome as seen in Uganda He believes that certain features are pathognomonic, especially the pancreatic lesion and the 'stellate" fibrosis of the liver He holds that atrophy of the acinar cells of the pancreas with marked decline in the extrinsic secretions of the gland is a fundamental feature of the syndrome and perhaps the essential and earliest lesion If Davies' observations and interpretations are correct, and if they apply to kwashiorkor as seen in other regions then the pathogenesis of the syndrome can be stated in some such terms as the following Under the influence of protein malnutrition which is acutely precipitated by weaning, the pancreas nd other organs of exocrine secretion are first affected This leads to decline in enzymatic and other efficiency of the gastrointestinal tract and creates a vicious circle of conditioned and dietary protein deficiency, which, in turn, affects the liver and every tissue in the body

Turning from Africa to other parts of the world the incidence of kwashiorkor was reviewed by Meiklejohn and Passmore[22] and has recently been brought up to date[28, 19b] A survey of kwashiorkor in Central America has been made by Autret and Behar and will be reported There is a general impression that kwashiorkor is a syndrome apparently basically the same as 'culebrilla" syndrome pluricarencial, "syndrome hipoproteinico' and related syndromes of Central and South America and the "fatty liver disease" of Jamaica Apart from the pigmentary disturbances of the skin which are usually present it is probably basically the same as the 'Mehlnährschaden' of Germany and Austria in the early 1900 s, and the 'dystrophie de farineuse and distrofia de farinacei' still recognized in the unpigmented or lightly pigmented children of southern Europe The report of the third session of the Joint Committee (November 1952) contains a comprehensive list of titles which the Committee believes to signify syndromes of the same nature as

kwashiorkor and covered by the general term "protein malnutrition" as defined in the report " a state of ill health occurring where diets are habitually poor in good quality protein, while they are more nearly adequate in calories It includes deficiency of protein foods, imbalance of amino acids, and deficiency of accessory food factors associated with protein metabolism It is most easily recognized when calorie intakes have been relatively high from the use of starchy foods "

Because no one name commands general acceptance to cover the "protein malnutrition" syndromes of the American tropical belt and because the Joint F A O/W H O Expert Committee on Nutrition has been inclined to include them under the heading "kwashiorkor," I have compromised in this communication by grouping them for convenience under the term "American kwashiorkor " I could not use the plain term "kwashiorkor" because on account of the apparent difference in sequelae, I have asked the question whether kwashiorkor and "American kwashiorkor" are the same disease? In spite of difficulties which will be discussed, I believe the answer to be in the affirmative.

A brief summary will be given of present knowledge in regard to the liver lesion or lesions associated with kwashiorkor This falls under two headings (1) the pathology of the liver in kwashiorkor in childhood, and (2) the liver lesions of adults in the areas of prevalence of kwashiorkor and protein malnutrition and the possible relationships between these adult lesions and those of the children Brock and Autret reported that the liver pathology of African kwashiorkor consisted of some combination of fatty infiltration, necrosis, and fibrosis These three features showed various combinations, and there is much experimental evidence that all three may occur separately and independently as manifestations of dietary deficiency The experimental evidence for the separate origins of fatty infiltration and necrosis are reviewed elsewhere in this monograph † There can be no doubt that the processes described under these two terms are quite distinct, but it should be emphasized that even the "dissolution of fatty cysts" described by Hartroft in his lipohepatosis is a form of death of cells It was in this wide sense that the liver lesion of kwashiorkor was described by Brock and Autret as "fatty infiltration cellular necrosis, or fibrosis " It was intended to leave the pathogenesis an open question The available evidence certainly suggests close analogies between the liver lesion of kwashiorkor and experimental fatty liver cirrhosis but it would be premature to press this comparison too far This subject is discussed further in the section on definitions (Appendix, p 709) Workers on kwashiorkor in Africa have inclined strongly to the concept of the independence of fatty infiltration and of fibrosis Both Gillman[13] and Davies[3] have recorded their views on this subject There seems to be no reason why the fatty infiltration of kwashiorkor should not be permanently reversible on provision of a proper diet, and the problem of continuing fibrosis is discussed in a later section of this communication.

Brock and Autret in their report stated that "it can be accepted as reasonably probable, from their world distribution, that cirrhosis and primary car

† See page 623

cinoma of the liver on the one hand, and kwashiorkor on the other hand, are aetiologically related The evidence strongly suggests that both are due to dietary deficiency, particularly deficiency of protein " The evidence for this statement is worth critical examination, since it is based on some tentative assumptions

The areas of the world in which kwashiorkor is prevalent are very similar to but not identical with the areas in which cirrhosis and primary carcinoma of the adult human liver have a high incidence The areas of correspondence are readily explainable on the attractive hypothesis that the gross hepatic damage of infantile kwashiorkor is the starting point of a fibrosis which proceeds insidiously throughout adolescence and presents itself clinically in the third and fourth decades of life as a "nutritional cirrhosis" which is often complicated by primary carcinoma of the liver This hypothesis is at present only provisional Careful examination of maps of the world areas of noncorrespondence between infantile kwashiorkor on the one hand and 'nutritional cirrhosis and primary carcinoma of the liver in adults on the other may yield important clues as to the reliability of the assumption

What is needed at present is a series of accurate maps of the world reflecting the areas of incidence of (1) kwashiorkor or related protein malnutrition syndromes, (2) prevalence of primary carcinoma of the liver, and (3) prevalence of cirrhosis of the liver The first map has been constructed from the literature[7, 22, 23] but will need to be filled in as reports accumulate (FIGURE 1) A version of the second map is contained in Berman[2] (FIGURE 2)

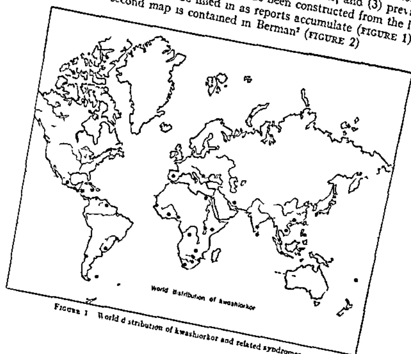


FIGURE 1 World distribution of kwashiorkor and related syndromes

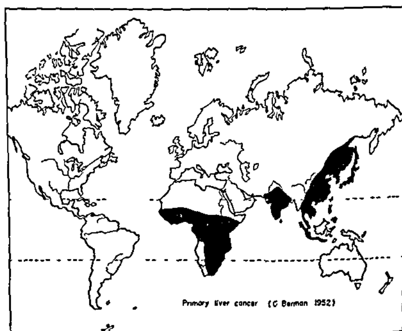


FIGURE 2 World prevalence of primary carcinoma of the liver

Without detracting from the value of Berman's work, this map can be criticized both artistically and scientifically in its "black and white" character The figures given in FIGURE 3, prepared by myself, show that the difference in incidence of primary liver carcinoma could be better represented by a gradual merging from black through grey to white as we pass from the tropics through the pigmented residents of temperate climates to the European races Berman's map can also be criticized on the score that he produces no evidence that the prevalence of primary carcinoma admitted to exist in South China continues up to North China as represented on his map

If these two maps are compared, there is considerable correspondence between the latter and the world map of kwashiorkor as far as Africa, India, China, and the Far East within the tropical belt are concerned but a notable discrepancy with respect to the tropical belt of the American continents Does this blank in the tropical portion of the map of incidence of primary liver cancer reflect actual conditions, or is it due to failure to record and report? Warvi[31] said, "Near and Far Eastern races have a greater incidence of liver cell carcinoma than those of Europe *or the Americas*" Berman's summary confirms this, and apparently there is no contrary evidence

I have sought unsuccessfully in the literature for information on this point and have questioned and corresponded with those who might know The

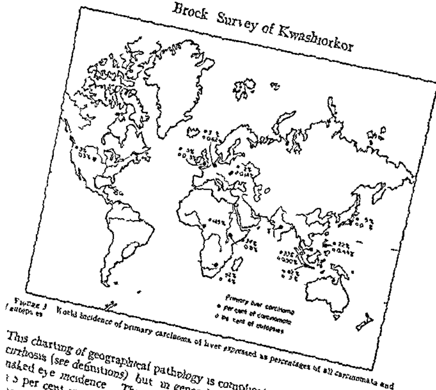


Figure 3 World incidence of primary carcinoma of liver expressed as percentages of all carcinomata and of autopsies

This charting of geographical pathology is complicated by different concepts cirrhosis (see definitions) but in general the figures represent macroscopic naked eye incidence The open circles represent incidence rates of less t 5 per cent in general autopsies and the black dots higher rates It will een that the difference between the tropical and nontropical areas is con tble but not as gross as would be expected from the map of primary liver cancer For interest in relation to later discussion I have also charted the areas of prevalence of siderotic cirrhosis and of liver damage resulting from hepatotoxic alkaloids contaminating food

The only data given for the American tropical belt are those derived from reports on the yellow fever viscerotome service a method whereby post mortem liver samples were obtained without doing an autopsy The figure of 2 per cent for Colombia represents 404 cases with signs of cirrhosis out of 22 000 specimens[12] That of 0 5 per cent for central and northern Brazil represents 55 livers with advanced cirrhosis out of 29 592 specimens[6] It must be remembered that these were all microscopic specimens If the data are based on the usual criteria the incidence of cirrhosis is low in these two large countries of the central American belt I have been able to find no pub lications on the cirrhosis rate for this area based on the usual autopsy inci dence rate

If microscopic criteria are taken for the diagnosis of cirrhosis then the in c lence becomes very much higher in some parts of the world The Gillmans[13]

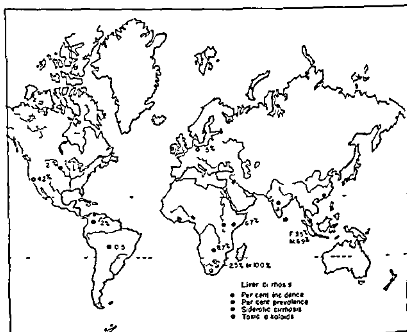


FIGURE 4 Liver c rrhosis expressed as a percentage of autopsies in areas of high and low Incidence Areas of liver damage associated with siderotic or iron rich c rrhosis and with tox c alkaloids are shown

for example, claim that among 261 adult African (Bantu) subjects dying "sudden and rapid accidental death" only 12 6 per cent of livers were histologically normal Such a figure cannot be compared with figures from other parts of the world since histological [illegible]

[illegible]

than his macroscopic rate of 2 5 per cent

On the other hand, Bauer and Kerr, although they record only 55 cases of "advanced cirrhosis' in 29,593 specimens, nevertheless record that, in 1594 of those specimens in which lesions of *S mansoni* were found, "periportal reaction" is always present and ranges from a slight hepatitis through various degrees of fibrosis with chronic inflammation to severe cirrhosis And in 2298 further specimens showing malarial pigment, there were 23 per cent with "periportal reaction " Admitting that periportal reaction is not synonymous with cirrhosis, it seems that the figure of 55 cases of "advanced cirrhosis" in the whole series represents a very conservative estimate of the actual incidence of cirrhosis in Brazil

The gap covering the American tropical belt in the world map of cirrhosis of the liver constructed from published information is very obvious, and again the question arises, as in the case of the map of primary carcinoma, as to whether this gap is real or only apparent? The opinion of some of those who have had experience is that it is only apparent Until it is filled in, it cannot finally be

oncluded that "American kwashiorkor" is the same disease as kwashiorkor in Africa, for it appears, at present, not to have the same sequelae

Interpretations

f there were any consequential relationship between kwashiorkor and pri y carcinoma of the liver, that sequence would presumably be through the insidious development of cirrhosis of the liver as a sequel to kwashiorkor, because (1) the correspondence between primary carcinoma and cirrhosis of the liver is so close and (2) experimental evidence suggests that protein malnutrition in animals can result in progressive and permanent cirrhosis

Two questions must be asked at this point (1) What is the evidence that kwashiorkor in African can produce permanent cirrhosis of the liver? (2) What is the evidence that "American kwashiorkor" can produce cirrhosis of the liver? It will be seen in the later discussion that a final answer cannot be given to either of these two questions

The next question to be asked is What other factors besides protein malnutrition may enter into the production of cirrhosis of the liver either as basal causative factors or as contributory factors? In the later discussion, this aspect of the problem will be treated under the following headings (1) viruses, (2) tropical parasites, (3) alcohol, (4) hepatotoxic alkaloids and other food contaminants, (5) high dietary iron intakes, and (6) genetic factors

The question will then be asked whether any of these factors can explain the gap in the prevalence of carcinoma of the liver in the Central American belt where "American kwashiorkor" is prevalent and where protein malnutrition is believed to be widespread

Finally the question will be asked whether kwashiorkor as seen in the area of world prevalence of primary carcinoma of the liver (i e , in Africa, India, China and the Far East) can be regarded as essentially the same as "American kwashiorkor"

Does African kwashiorkor produce cirrhosis? There has been a fairly general assumption that African kwashiorkor does produce cirrhosis largely because of the assumed geographical correspondence between kwashiorkor on the one hand and of cirrhosis and primary carcinoma of the liver on the other It must be emphasized, however, that this is only an assumption The only real evidence on the subject that has been put on record is the opinion of Davies[8] In discussing the pathology of the liver in kwashiorkor he states "The initial localization of the fat is in the periphery of the liver lobule, often sparing the cells of the lamina limitans As the disease progresses, the fat appears nearer and nearer to the central vein until the whole lobule is full of fat With the increase in fat there is an infiltration of the portal triads by round cells, and fine fibrosis appears around the periphery of the lobule, spreading out in a stellate fashion from the portal triads The fibrosis results from a thickening of the reticulum around the peripheral cells It spreads throughout the liver until in severe cases a state resembling a Laennec's cirrhosis is seen If the child survives either with or without treatment, the fat tends to disappear slowly, first from the centrolobular region, and lastly from the periphery, leaving behind a fine stellate fibrosis and round cell infiltration in the portal triads Treat-

ment with protein leads to rapid fat reduction and a flooding of the sinus with lymphocytes, but does not affect the fibrosis and cell accumulati These may persist for many years, if not for life, and in an African appea be a "hallmark" of kwashiorkor Why they should be so persistent, whe is well known that in other conditions quite well marked fibrosis of the l can disappear, is a mystery, but it suggests that there is continuing l damage of some sort " If this view is correct we must ask what the natur the "continuing liver damage of some sort" referred to at the end of Dav quotation is Is it continuing protein malnutrition, or do other cirrhotig factors which will be discussed below play a part? No other definite evide for the progressive evolution of cirrhosis of the liver out of African kwashio has been recorded in the literature The Gillmans[13] say, "We have very li information about the reactions of the livers of Africans between the age six and eighteen years, because during this age period, for some still unde mined reason, temporary but acute episodes of nutritional disease are r Biopsy in one seven year old child revealed that the increase in hepatic i may commence at this age We know most certainly that the hepatic i content increases rapidly after the age of eighteen years As we have indica primary hepatocellular carcinoma may and frequently does supervene betw the ages of sixteen and twenty years and may occur at an even younger ag In other parts of the world, *progressive cirrhosis of the liver does not appea be an inevitable sequel of syndromes resembling kwashiorkor* No evide has yet been published that the Mehlnahrschaden and starch dystrophy Europe progress to cirrhosis of the liver In the following discussion, it be seen that "American kwashiorkor" has not been shown to be associa geographically with a high incidence of adult liver cirrhosis My colleag Doctor P Suckling and Professor J G Thomson in Cape Town, have laborated with me in searching in biopsy material for continuing evide of cirrhosis of the liver in children recovered from kwashiorkor among the C colored (non Bantu) population of Cape Town (Brock, 1949) So far,

cirrhosis of the liver after it has been cured and provided that the subseq diet is satisfactory and cirrhotigenic agents are not prevalent On the o hand, there is very convincing evidence that, in certain parts of Africa, at le fibrosis may persist for many years, if not for life, after clinical recovery f kwashiorkor in the presence of "continuing liver damage of some sort" "increase in hepatic iron "[13]

Does American kwashiorkor produce cirrhosis? There seems at present be very little, if any, evidence that American kwashiorkor produces cirrh In fact, the absence in the literature of evidence for a high prevalence of mary carcinoma of the liver and of cirrhosis of the liver in the American trop belt may be regarded as indirect evidence against such a supposition matter must, however, be left open until further evidence accumulates further accumulation of evidence should confirm that these two disorders

not prevalent, we must conclude either that "American kwashiorkor" is not the same as African kwashiorkor or that the "continuing liver damage of some sort" postulated by Davies in Africa is not prevalent in the American tropical belt

What other factors besides protein malnutrition enter into the production of cirrhosis of the liver? One of the few things that are certain about cirrhosis of the liver is that it may result from several, if not many, causes In all probability, the causation is frequently multiple I will not examine the evidence on experimental nutritional liver damage in animals since it has been so thoroughly discussed in this monograph Turning to the human being, I do not think anybody will dispute the role of malnutrition in the production of human liver damage What is more relevant to our present discussion is to ask (1) Can any form of human malnutrition lead to a fibrosis which is permanent or progressive when the diet is restored to normality and when other cirrhotigenic agents are absent? (2) Can the effects of severe human malnutrition, as in kwashiorkor, be continued throughout the rest of life by milder degrees of malnutrition in the absence of other cirrhotigenic agents? (3) What other cirrhotigenic agents need be seriously considered in the causation of the cirrhosis which is so prevalent in the areas of high prevalence of primary carcinoma of the liver? The only reply that can be made to the first question is that there is, at present, no convincing evidence in the human being, and there is unlikely to be in the future The answer must be sought by analogy from animal experiment To the second question the reply must be made that both animal experiment and the study of the epidemiology of kwashiorkor suggest strongly that continuing protein malnutrition may be an important factor in determining the progressive evolution of adult cirrhosis out of kwashiorkor But if this assumption is true, why does it not also hold for the American tropical belt? In the light of this noncorrespondence, we must assume either that protein malnutrition is not continued into adolescence in the American tropical belt to the same extent or in the same form as in Africa, or else that cirrhotigenic agents other than malnutrition operate to produce adolescent cirrhosis in Africa but not in central America

The possible or probable cirrhotigenic agents will now be discussed in order

(1) Viruses There can be no doubt that viruses may act as cirrhotigenic agents It may well be argued that they act only as cirrhotigenic agents when other cirrhotigenic factors including nutritional deficiency are simultaneously operative This view might well be supported, but the fact remains that viruses can be regarded as related to certain forms of cirrhosis in as definite a way as the tubercle bacillus may be regarded as related to tuberculosis It is likely that viruses if they produce progressive cirrhosis do so because they become established in the liver over a long period of time

(2) Tropical parasites have been blamed in the past for cirrhosis of the liver, but their etiological role seems to be far less definitely established than is that of the viruses Schistosomiasis might be picked out as the tropical parasite for which there is most evidence of a cirrhotigenic action The fact remains, however that wherever schistosomiasis is prevalent malnutrition is also prevalent, and there is no satisfactory evidence that any tropical parasite,

and the Gillmans' "cytosiderosis" concept. It is felt that the use of the term "haemochromatosis" by Strachan[27] and by the Gillmans[13] for the siderotic cirrhosis of the Bantu people of southern Africa is confusing since the balance of evidence suggests that these cases are quite distinct from classical haemochromatosis.[25]

Primary liver cancer or primary carcinoma of the liver. These terms are used interchangeably and are preferred to "hepatoma" and "hepatocellular carcinoma" in this communication because the former terms are wider and the precise cellular type of carcinoma is not always specified in reports from the areas of prevalence. In all probability, however, the prevalent condition is usually a hepatocellular carcinoma.

Pellagra. The term "infantile pellagra" has been specifically rejected in favor of kwashiorkor, and a few words should be said about its use in relation to the prevalent clinical syndrome in adult Bantu subjects in South Africa which is linked by the Gillmans[13] with the siderotic cirrhosis which they call haemochromatosis. They do not define the "pellagra" they are describing except in the negative sense: "We are of the opinion that, unless the dermatosis is present, then the nutritional syndrome should not be regarded as pellagra." They clearly recognize the possibility that pellagrous dermatosis may occur in several different patterns of complex metabolic disturbance resulting from different dietary patterns, but use the term indiscriminately for them all. We see, in Cape Town, pellagra in Cape colored people, of mixed but predominantly non Bantu blood,[4] which is indistinguishable from the classical pellagra described in Southern Europe and the Southern U. S. A. We see also "pellagra" in Bantu subjects which is apparently indistinguishable from the "pellagra" described by the Gillmans. In our experience, the disease in these two different races is very different and it is confusing to call them both pellagra. I prefer to call the latter variety "Bantu malnutrition with pellagrous dermatosis." At least it ought to be called "Bantu pellagra" to distinguish it from the classical type. To mention only a few of the differences, pellagra in the Cape colored people is not associated with siderotic cirrhosis nor with any increased susceptibility to primary carcinoma of the liver. The same is true of classical pellagra in Southern Europe and Southern U. S. A. "Bantu pellagrins" in South Africa, on the other hand, have a high incidence of siderotic cirrhosis and primary liver cancer.

Necrosis. Experimentally fatty liver cirrhosis and acute necrosis of the liver appear to be quite distinct processes as is evidenced by the division of the proceedings of this conference between these two headings. There has been a tendency among experimentalists in dietary liver damage to confine the term 'necrosis' to the acute necrotic variety instead of using it in its wider meaning of 'death of cells' from any cause. This trend may be useful although it is etymologically dubious. It is necessary, therefore, to emphasize that Brock and Autret in their report used the term in its wide sense and did ...which

regard to kwashiorkor undoubtedly ... experimental

fatty liver cirrhosis, but the analogy cannot be pushed too far This is particularly important in respect to adult "nutritional cirrhosis" In discussing this subject Himsworth[18] says 'massive hepatic necrosis and its sequelae are unusually common in tropical and subtropical races subject to malnutrition" The factors, other than diet, which may be involved in the production of tropical cirrhosis are categorized in the text Some of these are known to cause acute necrosis of the liver

Postscript

After hearing the discussion at the conference on which this monograph is based, I think it is necessary for me to emphasize that my personal experience of kwashiorkor is limited to the African continent, but includes the material seen in Johannesburg and Pretoria where the syndrome is seen in Bantu children who have usually not been exposed to tropical parasites, and in Cape Town where among Cape colored children there has been no opportunity for exposure to tropical parasites

With regard to the comparison with "American kwashiorkor, I am handicapped by the fact that I have not yet seen the report by Autret and Behar My impression from the published literature and from pictures shown is that American kwashiorkor' at least in cities is often more dominated by undernutrition than is the case in what has been defined as "protein malnutrition, especially as exemplified by the pictures in the monograph by Brock and Autret Obviously, there are in all areas all degrees of transition between protein malnutrition and undernutrition

Conversations at the conference confirm my impression that cirrhosis and primary carcinoma of the liver are far more prevalent in the American tropical belt than the literature indicates

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Discussion of the Paper

DOCTOR SCHWARZ I wish to congratulate Dr Brock on his very outstanding review and on the remarkable achievements which he, in collaboration with other members of the Joint F A O/W H O Expert Committee on Nutrition, has accomplished May I be permitted, however, to remark, that to my knowledge there is no conclusive evidence that kwashiorkor is just a protein deficiency I may be wrong, but it seems advisable to sound a warning against the general acceptance of this idea For *practical* purposes, particularly for indoctrinating and training less educated populaces, the designation of kwashiorkor as a 'protein" deficiency might be of value This, however, should not distract the attention of a scientific forum from the fact that the therapeutic benefit derived in a deficiency disease from protein foods or even from an (impure) protein itself, like casein, does not necessarily indicate that this disease is caused by lack of "protein" as such The effect can equally well be due to trace factors contained in these proteinaceous materials, or also from one individual protein building stone The quantitative separation of vitamins from proteins is extremely difficult Finding or preparing vitamin free proteins for vitamin free diets has been one of the most serious obstacles in vitamin research during the last 50 years

We know that, with the exception of fat soluble vitamins all important dietary factors may occur in nature in more or less firmly bound forms In any cases autolytic enzymes are required for their liberation and in some stances, for example in the case of biotin, hydrolysis with strong acid must

be used to achieve complete release. Still fresh in our memory is vitamin B_{12} which not long ago was called "animal protein factor." This monograph contains two further examples of specific nutritional factors which occur strongly bound to protein. Both of these concern the liver. The xanthine oxidase factor which after prolonged attempts at purification and isolation from plant proteins has now been disclosed to be a trace element, molybdenum,[1] and Factor 3 against dietary necrotic liver degeneration which is obtained in concentrated form from casein and which is present even in so-called vitamin free caseins.[2]

Taking these observations into account and realizing further that not all of the factors protecting against fatty liver and cirrhosis have been identified as yet, I think it would be fair to say that one should be reluctant to label kwashiorkor as 'protein deficiency,' at least as long as it has not been shown to be cured by pure amino acid mixtures.

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THE PATHOLOGY OF DIETARY LIVER DISEASE IN TROPICAL AFRICA

By J N P Davies

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The only form of dietary liver disease in tropical Africa about which it possible to speak with any precision is the extreme fatty infiltration which seen in kwashiorkor In a florid case of this disease (*e g* , in a child of about tv years of age), the liver, while not very much enlarged, is a tawny yellow buff color or, in a very anemic child, may be stone colored, due to intense fat infiltration In older children, some degree of increased fibrosis of the liv may be appreciated Not all children with kwashiorkor have such a degr of fatty infiltration, for in some there may be little or no fat

The changes in the liver in kwashiorkor, as shown by biopsy and autop studies, go through a sequence which results in permanent alterations to t structure of the liver In West Africa, attention has been drawn (Auffret a Tanguy, 1950, Silveira and Jelliffe, 1952) to the presence of gross fatty infilt tion of the liver in very young children Such lesions are not common encountered among young children in Uganda and where there is any a *preciable degree of fatty change seen in the infant liver, it is usually* centi lobular in distribution So far as my experience goes the livers of new bo African children and of neonates in Uganda do not differ appreciably from t livers of European children Apart, therefore, from the presence of malai pigment, which after a while acts as a convenient delineator of the boundari between liver lobules, the African liver in those first few months of life, wh the child is growing well (Welbourn, 1951), presents no significant change [illegible]

first at the periphery of the liver lobules This is always the case in childr where there is no precipitating factor, *e g* , tuberculosis In complicated cas the initial fat deposition may be centrolobular or irregularly sited, varying from lobule to lobule Where this has been recognized by a study of biopsy material, the attention of clinicians has, on several occasions, been drawn to the presence of a hitherto unrecognized infective condition (Davies, 1948, 1950)

Provided there is no such complicating factor, the initial locus of the fat deposition is in the cells at the periphery of the liver lobule, though the cells of the lamina limitans round the portal triads often remain free of fat for a considerable time As the clinical condition deteriorates the peripheral fat droplets increase in size and the cells down the sinusoids toward the central vein are progressively involved till in a severe case, practically every cell is full of fat and some small fatty microcysts may be seen FIGURE 1 shows a part of two liver lobules with a portal triad and two central veins with fatty infiltration of the liver cells the larger lobules of fat being in the outer parts of the lobule Dark malaria pigment is seen also, collected chiefly at the periphery

With the infiltration of the fat into the liver, certain other changes are noticeable The lymphocytic infiltration of the sinusoids has been mentioned but these cells and many others become concentrated in the portal triads The most plentiful cells are lymphocytes, but plasma cells and macrophages and neutrophil and eosinophil polymorphonuclear cells may also be present the latter two types being plentiful in cases complicated by a septic infection or ancylostomiasis

Such notable collections of cells in the portal triads are seen in FIGURE 2 As you can see, there is a considerable degree of fatty infiltration with the most intense fatty deposition in the peripheral cells of the liver lobule The portal triads are crammed with cells and they are also present to some extent along the periphery of the lobule The cellular infiltration of the portal triads seems to be one of the persisting alterations in the liver because, except in the early months of life, it is very rare to see an African liver which does not contain an excessive number of cells in the portal triads In addition, comma shaped nuclei are present in fair numbers in the portal triads, and these are probably the nuclei of fibroblasts They do not seem to occur, in the early stages at least, outside the portal triads

The third change that takes place is in the reticulin framework in the periphery of the lobule The fibres thicken and split and reduplicate This change takes place round the peripheral cells and is most marked at an early stage near the portal triads, but later it is apparent round the whole of the periphery of the lobule The lamina limitans cells are separated by the thickening reticulin and they and the peripheral cells appear to undergo a process of strangulation for some of them disappear with collapse of the reticulin framework The reticulin undergoes collagenization, so at an early stage, the lobular outline is accentuated not only by the malaria pigment, but also by the reticulin thickening and collagenization FIGURE 3 shows such fibrotic changes The liver is moderately fatty, the portal triads are being joined one to another by the reticulin thickening and collagenization This accentuation of the lobular pattern of the liver is never so marked in children we have studied that it could be called a cirrhosis and these changes all occur round the lobule periphery In a few cases, the collagenization is so marked that all the portal triads are linked together by fine strands of fibrous tissue, more reminiscent of the liver of a pig than a normal human liver In his studies of the fatty liver in cases with tuberculosis, Saphir (1929) remarked on the development in a small percentage of his cases of such a true unilobular type of fibrosis

In time, should the child survive even without treatment by a high protein diet, the fat will diminish and slowly disappear from the liver even without any marked clinical improvement, and it can be removed more speedily by feeding a high protein diet Under both circumstances, the fat diminishes and disappears first from the centrolobular regions and last of all from the peripheral cells As it leaves the fibrosis often seems to increase and become more apparent The cells after the fat has left, exhibit the same hypocytoplasmic appearance as before it appeared Though the reticulin thickening and fibrosis seem to start with the fatty infiltration, the relationship is certainly not simple It is a time relationship rather than a causal relationship

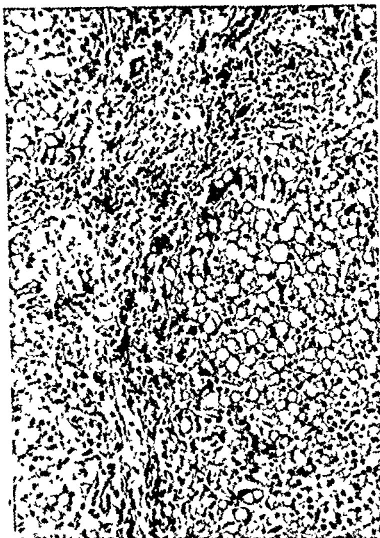


Fig. 2. Liver of child with kwashiorkor showing fibrosis, pigment and cell infiltration at the periphery of the lobule where the fat infiltration is maximal. H. & E. stain. ×210

Sometimes there is severe fat infiltration with minimal fibrosis, sometimes marked fibrosis with little fat infiltration, and the cause of the fibrosis awaits elucidation, particularly as Waterlow (1951) has shown in West Africa that a similar type of fibrosis can develop in the liver without fatty infiltration. Our experience leads us to suppose that there are at least two sets of causal factors



FIGURE 3 Liver of ch ld with kwash orkor show ng gross fatty infiltrations The early peripor spread ng from the portal tr ad can be seen H & E sta n X210

operating one leading to the fatty infiltration, the other to the fibro The net result is however that the vast majority of Africans, both adı



FIGURE 4 Biopsy specimen of African child's liver showing stellate fibrosis of the portal triad extending to delineate the lobular boundaries Reticulin stain ×150

the next along the boundary of the lobule FIGURE 4 taken from a liver biopsy shows this lesion which I call a 'stellate fibrosis' The smooth outline of the portal triad and the lamina limitans are disrupted by these fibrous scars in the vast majority of African livers I once suggested (Davies 1948) that these lesions were the 'hallmarks" of kwashiorkor In Uganda, they are not

found before the weaning period and they are almost universal afterward so that some changes take place in this period that mark the period if not the disease

It would, I think, be most unwise for me to speculate on the possible causes of the fibrosis I do not think the fibrosis is a result of the fatty change but whatever the cause the stellate fibrosis is a persistent lesion and I think it continues in a slow, stealthy fashion to increase in the absence of fat from the liver Nor have we any very clear ideas on the exact causes of the fatty infiltration except a very strong suspicion that it is somehow linked to the atrophy of the pancreatic acinar cells and the other cells which lose protein by enzyme secretion The histological evidence we have accumulated suggests that the pancreatic atrophy precedes the liver changes and that the fatty infiltration of the liver is a transient though important episode in the disease The primary involvement of the pancreas has also been shown by Veghelyi (1951) But the precise connections between the pancreatic atrophy and the fatty infiltration of the liver have not been discovered, and to discuss them would take me outside my field

It is more important to discuss the relationship of the changes in kwashiorkor to disease of the liver in later life We are most ignorant about the condition of the liver in the later years of African childhood and the earlier years of adolescence But during this period, and indeed for the rest of his life the African is eating a diet qualitatively little better than that on which he developed kwashiorkor A diet low in protein, especially animal protein and high in carbohydrate is all too common Yet cirrhosis of the liver is rarely seen in childhood in Uganda nor in the early years of adolescence Puberty with its growth spurt does not bring out a fresh crop of kwashiorkor cases and we do not know why this is so Perhaps puberty in Africans is more delayed and the growth spurt not so marked We know that the onset of menstruation is delayed Moreover, we must remember that the endocrine changes

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much early work on this disease was largely devoted to the study of adult cases The atrophy of the pancreas and of the intestines is just as marked and there is the same fatty infiltration of the liver though this is not so extreme The major difference lies in the much greater amount of fibrous tissue present in the liver, a considerable increase in the stellate fibrosis In some cases irregular strands of fibrous tissue cut off groups of cells at the periphery of the liver lobules Both clinically and pathologically, some cases merge into Laennec cirrhosis but the connection between these states remains obscure The gross fatty infiltration seen in children is not very frequently encountered in adults and we have no evidence to suggest that our cases of Laennec cirrhosis have gone through a long continued stage of fatty infiltration in adolescence Iron pigment is not present in unusual amounts in our cases and the only pigment seen is a brown perinuclear pigmentation apparently due to a lipochrome substance

Laennec cirrhosis is quite frequently encountered in Africans Among 2/1

autopsies there were 115 cases as well as 74 cases of postnecrotic scarring Analysis of these cases has shown (Davies 1952) that, contrary to expectations,
[illegible]

and probably to protein malnutrition

I shall, if I may, conclude this brief presentation by summarizing our main problems as I see them The frequency and importance of kwashiorkor is now well recognized The basic lesion seems to be a selective atrophy of the enzyme secreting glands affecting the pancreas the small intestines, and the salivary and lacrimal glands We do not know the precise cause of this atrophy At an early stage in the disease there is an intense but transient fatty infiltration of the liver, the fat droplets occurring first in the peripheral cells of the liver lobule, which are the last to be freed of fat We do not know the cause of this fat infiltration, its relation to the preceding enzyme cell atrophy, or the factors that enhance its removal from the liver With the fatty infiltration there occurs a flooding of the portal triads and to a lesser extent the sinusoids with inflammatory cells the lymphocytes being especially prominent We do not know what they are doing There is also the reticular thickening and the fibrotic process near the portal triads and around the lobule periphery The cause of this awaits elucidation We do not know how this continues to progress in the absence of fat from the liver, the significance of this progression its extent, or its relation to Laennec cirrhosis We do not know the cause of the great frequency of Laennec cirrhosis but we do not think that it is a sequel of infective hepatitis We also do not know the causal factors of carcinoma of the liver although we suspect them to be endocrinal Malnutrition, liver disease, and hormonal alterations seem to be fundamental to our understanding of disease in Africans They are being investigated by only a few widely scattered scientists

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CLINICAL ASPECTS OF THE TREATMENT OF KWASHIORK

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TERMINOLOGY AND ETIOLOGY

Although this paper discusses the clinical aspects of kwashiorkor, it i of all necessary to discuss briefly etiology, for this determines the appro the problem of therapy There is now general agreement that a di clinical and pathological disease process, called kwashiorkor, is very co in certain tropical countries This condition occurs mainly in young ch and follows the prolonged consumption of diets which have lacked variet in which the proportion of protein, especially that of animal origin has low, whereas the proportion of carbohydrate has been high These diets a have rather a low protein calorie ratio and the animal protein calorie ra always very low It is considered that this unbalanced diet was usually in amounts sufficient to satisfy the child's hunger, but more informat desired on this important point At a certain stage in the illness, ap decreases and anorexia begins, so that a decreased intake of this unbal diet often then occurs (Welbourn, 1953) At this stage of the illness, th also impaired digestion and utilization of all foods The intake of fats i usually low Children who develop kwashiorkor have been receiving or no milk, either of human or animal origin, and very little protein of a origin The condition is therefore treated, cured, and prevented by g diets rich in protein I should also make it clear that certain of my colle in Uganda consider that older children and adults may occasionally from a similar or an allied disorder, but this paper is confined to a stu the disease in young children, among whom the condition is far more co because of their high requirements for protein and allied substances

Kwashiorkor is one member of a big group of diseases referred to as th eases of protein malnutrition It would appear unwise to speak of kwash as merely "protein deficiency" for there are many varieties of protei ficiency even among children For instance one type of protein defic occurs in nephrosis and it is quite distinct from kwashiorkor In additi is quite uncertain how far calorie intake, total protein intake, one or amino acids, or vitamins are responsible as well as other substances k or unknown but concerned with animal protein or with protein metab It is also not known why an adequate supply of human breast milk, whic rather the low protein calorie ratio of 1 5 gm to 100 calories is so highly tective against kwashiorkor (Gyorgy, 1952)

A noncommittal term such as kwashiorkor (Williams, 1933), is ther preferred In many areas of Africa in which there is little cows' milk an protein rich cereals the peasants have noticed that healthy, breast fed b often develop a serious illness shortly after complete weaning In r tropical countries as in Europe until Elizabethan times breast feeding

* Thanks are due to the Director of Medical Services Uganda for permission to publish this paper

continue for two or three years and often with considerable benefit This was often possible in Africa under the conditions of marital life which were a part of polygamy, but conditions and customs are changing rapidly If a

people of the Gold Coast, among whom Doctor Cicely Williams was working when she published her accounts of this disease (1933) Other tribes have other names and about fifty different terms have been employed by different doctors These names often refer unfortunately to multiple deficiencies, possibly those of the vitamins, and these terms have crept into the literature, especially that of South America Yet treating these cases mainly by multiple-vitamin therapy often results in fatalities

Recently it has become clear that Czerny & Keller (1906) were describing an identical condition in Germany under the name of Mehlnahrschaden Their description of the disease, however, was very incomplete No changes were noted in the pigment of the hair and the pancreatic lesion was not described so that subsequent workers, such as Véghelyi (1948, 1950), who almost re discovered the condition in Hungary and called it "nutritional edema," were unable to identify this disease with Mehlnahrschaden The old and correct teaching of the German pediatricians was kept alive and developed in Italy, where Frontali (1952) has confirmed the presence of the pancreatic lesion and the hair changes in this disease (called starchy food dystrophy" in that country) This disease is present in nearly 5 per cent of children in Southern Italy The presence of the characteristic 'enamel paint dermatosis' and "flexural fissures" of kwashiorkor has however not been described in any case of Mehlnahrschaden, or "starchy food dystropy"

In the literature of the United States a few cases of kwashiorkor probably have been described, such as that of McEnery (1933) It is difficult to evaluate the more numerous reports on the cases of 'nutritional edema" seen in young children in the United States and in other countries In cases of "nutritional edema" in infancy, there has not been described the dermatosis the fissures, the dyspigmentation the pancreatic lesion, or the high mortality, which are characteristic features of kwashiorkor "Nutritional edema' was ascribed at one time to protein deficiency and it is probable that many cases of this disease, especially among young children, were cases of kwashiorkor, and were not cases of hunger edema due to calorie deficiency, or beri beri due to thiamine deficiency, all three of which are different varieties of "nutritional edema" The latter is not a suitable term for kwashiorkor, for some severe cases show no edema and mild cases of kwashiorkor never show any edema

probably very common in many tropical countries It is, however, difficult to define (FIGURE 1) Thus, Welbourn (1953) has found signs suggestive of mild kwashiorkor in 46 per cent of the children attending child welfare clinics near Kampala, Uganda Wherever possible, treatment and prevention of

the milder disease should be by vegetable protein, for this is cheaper and more plentiful

The Clinical Picture of a Typical Case of Severe Kwashiorkor

This is so distinctive (Trowell, Davies, & Dean, 1952) that a diagnosis can usually be made as soon as the case is seen (FIGURES 2 and 3) The child is seriously ill and is usually from 9 to 36 months of age, although typical cases can occur outside this range The weight and height are always very subnormal Mental apathy is always present and peevishness on being disturbed is usually noted The appetite in advanced cases is always very poor The abdomen is usually distended Undigested food is passed in bulky or loose stools, which are usually frequent Diarrhea may be very severe or absent and depends on the food eaten Subcutaneous fat is often well preserved during the early stages but is lost later if life is prolonged The muscles waste early and severely in this disorder Edema is usually present, but may be absent or slight in cases otherwise typical Edema tends to be generalized, the feet and face being involved early *Effusions into serous sacs are absent or slight so* ... failure ... Casts ... s shows some change in its texture, becoming soft and straight It is easily shed or pulled *out of the scalp* *Some change and loss of hair pigment* is usually but not always, seen, but this change may only be indisputable in those whose hair previously was black Dyspigmentation, however, occurs in all races whatever the color of the hair Some change in skin pigmentation may occur Generalized hypopigmentation especially obvious around the mouth may be seen in Africans Races with a white skin usually show no change but generalized hyperpigmentation has been occasionally described

The various eruptions on the skin are complex and should not attract too much attention Like the changes in the mucous membranes they are essentially variable Usually they are only seen as a terminal condition in very advanced cases They are not present in all cases even at death The only dermatosis which, in my opinion is peculiar to kwashiorkor is best described as "enamel paint dermatosis' (FIGURE 4), or "erosive dermatosis" since, although originally likened to the 'crazy pavement" of an old English garden path, this is not a happy comparison and the latter term has been used to describe several different skin lesions "Enamel paint dermatosis' appears almost exclusively on areas exposed to much pressure, especially if this is combined with moisture, any discharge, or the sweat which is common in the tropics This dermatosis has a definite evolution At first, small purple stains darken rapidly into hyperpigmented, raised, burnished plaques and these appear first in the inguinal and napkin areas and over the bony prominences of the legs trunk arms and head (FIGURE 5) These plaques have a sharply defined linear edge They enlarge rapidly and coalesce, then proceed to loosen as with some underlying moisture and peel thus disclosing pale eroded, moist areas of raw skin These plaques never appear in response to sunlight, although in many respects they resemble the eruption seen in certain



FIGURE 1



FIGURE 2



FIGURE 3

FIGURE 1 (left). African child with mild kwashiorkor: underweight, fair nutritional status, much loss of hair pigmentation. Good gain of weight and black crisp hair appeared after many weeks on skim milk. FIGURE 2 (center). African child with severe kwashiorkor showing wasting, edema, dyspigmented hair, and fissured corner of mouth. FIGURE 3 (right). African child with severe kwashiorkor showing dyspigmented hair and skin; also edema.

cases of acute pellagra At times, erosions may occur in any of these areas with little in the way of preceding plaques These erosions are easily dismisse as areas of intertrigo, which indeed they are At the same time, in a few a vanced cases, very deep fissures occur in the flexures, especially if sustaine flexion of a limb causes pressure on the crease in the skin "Flexural fissures, therefore, appear in the armpit, front of the elbow, back of the knee, and be hind the pinna (FIGURE 6), and at the angle of the mouth, where they resembl angular stomatitis, but they are deep fissures which bleed and never appea sodden "Enamel paint dermatosis," "erosive dermatosis," and "flexur fissures" have not been described, as far as I am aware, in any other diseas All commence healing within a few days, if not hours, after giving skim mil protein

Large areas of glazed and "crackled skin" may occur in areas exposed t irritation and to dryness This skin shows little tendency to desquamat spontaneously in large plaques unless the condition is very extensive and subsequently well treated This condition, often called "mosaic skin o "crackled skin," would appear to be largely a nonspecific sign and may occu in many states of undernutrition and responds to an increase in the diet I my opinion, these conditions tend to be more severe in any disease of protei malnutrition than in states of undernutrition

In addition, a pellagrous eruption, even with vesiculation, may occur a the periphery of the limbs on areas exposed to sunlight and to wind It most common in children taking much maize, but it can occur on other diet This dermatosis only occurs as a terminal manifestation a few days or weel bef

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(Gillman & Gillman 1951), or in Italy (Frontali 1952), where it is accompanie by hyperpigmentation of the exposed areas The latter investigator has show that this dermatosis responds rapidly to *small* doses (large amounts are ver dangerous) of niacin and only very slowly to skim milk powder, which, howeve contains small amounts of tryptophan and of the Vitamin B complex Th explains why cases at one time were considered to suffer from "infantile pe lagra ' It must also be admitted that transitional conditions between "en amel paint dermatosis ' pellagrous dermatosis and "mosaic skin" are fre quently seen These three skin conditions appear to be allied in many way They all appear in response to some external injurious agent They tend to b hyperpigmented They all tend to desquamate, although to different degree and at different rates

It is not for me as a clinician, to say much about the stellate fibrosis whic in Uganda affects the portal traids and appears first in certain cases after th fatty liver of acute kwashiorkor in childhood This is described by my co league Professor J N P Davies in this monograph (page 714) One thing certain and that is that this stellate fibrosis very slowly increases during lat childhood adolescence and adult life while the patients show no signs severe kwashiorlor but taken as a group they continue to receive the sam diets with a low protein calorie ratio The diets do not differ from tho



FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 4 (above). African child with severe kwash[illegible]

taken by infants It is only the requirements which have altered In i opinion, these persons often continue to show signs suggestive of mild kwa iorkor, that is to say, they are underweight and have dyspigmented hair a a slightly low serum albumin and a high gamma globulin Frank cirrhos s the liver, unassociated with alcoholism or manifest tropical disease, is ve common in Uganda, even in young adults in the third, fourth, and fifth decad In my opinion, progressive stellate fibrosis often leads to frank cirrhos s Uganda, and all varieties of transitional forms are seen Acute necrosis the liver, in any form, is very rarely seen in children in Uganda It occ in adults and may lead apparently to cirrhosis It is impossible and unde able to do more than mention necrosis here, largely because necrosis of t liver has been reported as occurring in kwashiorkor This is extremely dou ful, except possibly a necrosis of individual hepatic cells, and almost no ref ence can be traced to this in any original communication on kwashiorkor

It is not proposed to devote any further time to a consideration of the ot signs of vitamin deficiency These are seldom seen except in the termi states of kwashiorkor, and it should be noted in passing that signs of vitar deficiency seldom occur in children or adults dying of general undernutri due to calorie deficiency Vitamin A deficiency appears to be very common cases of kwashiorkor in Asia Riboflavin deficiency is severe on maize die Both scurvy and rickets are said to occur, but are rare, especially the latt in most areas Thiamine deficiency has not been demonstrated in Afri but is considered to be present in some cases in Italy (Frontali, 1952)

It will be noted that, in all this description of the clinical state, no referei has been made to the liver In many cases of kwashiorkor, the liver does i appear to be enlarged during life, and there is little data to suggest that degree of enlargement has been assessed in cases coming to autopsy Uganda, it is seldom enlarged during life or at postmortem Abdomi distension may obscure enlargement On the other hand in certain parts the world, moderate enlargement is often detected during life (Waterlow, 19 Altmann, 1948) and I have seen cases showing enlargement in Kenya Sudan, Nigeria, the Gold Coast, and South Africa If, however, the liver any severe case of kwashiorkor, whether enlarged or not, is biopsied, mark fatty infiltration is almost always seen In noninfected cases, this commen first, and disappears last, at the periphery of the lobule, that is, near the por vessels Any direct transition from the fatty liver of acute kwashiorkor n frank cirrhosis (meaning gross disorganization of liver architecture by fibr tissue and nodules of hyperplasia) has never been recorded in any part of world in kwashiorkor in infancy

It is perhaps as well to close this description of the clinical picture by noti that the majority of cases of kwashiorkor in tropical regions have some fection present

Many of these such as the hookworms, deplete the body of proteins a increase the dietary requirements In the majority of cases, in my opinic it is very doubtful if these tropical infections play any significant part in pi ducing kwashiorkor and recent work (Welbourn 1953) suggests that, even the tropics bouts of respiratory infections are the commonest diseases

precipitate an attack of severe kwashiorkor In many cases, these infections

are severe or produce fever, it is essential to treat them while administering suitable dietetic treatment

This clinical picture would not be complete without reference to the biochemical changes These are regarded as constant features and it is desirable that communications on this disease should include biochemical data The most constant changes are a marked reduction in the serum albumin, in all the pancreatic enzymes (Thompson & Trowell, 1952) and in the plasma esterase and plasma lipase (Srinivasan & Patwardhan, 1952), in serum amylase, cholinesterase, and alkaline phosphatase There are also a reduced blood urea total cholesterol, and ester cholesterol (Dean, 1952b) Feeding with protein rich diets produces a characteristic biochemical pattern of recovery, which is

glands, especially the suprarenal (Davies, 1952)

The Treatment of Cases of Severe Kwashiorkor

The mortality rate in untreated cases with edema and anorexia is almost 100 per cent, and little improvement was effected until pathology and biochemistry had afforded some insight into the mechanisms involved In the tropics, at one time, these cases were ascribed entirely to some tropical infection or to congenital syphilis, and treatment remained unsuccessful until the diets were also improved

was erroneously focused on the vitamins especially those of the Vitamin B complex and on liver extracts No serious advances were made in treatment until pathology (Davies 1948) afforded some insight into the difficulty of digesting food, and experimental work, largely in the United States and in Europe focused attention on the fatty liver Although the use of known lipotrophic factors has on the whole proved disappointing, yet workers in the tropics felt that this disease was probably an aspect of protein malnutrition The detection of the pancreatic lesion of the reduction in their enzymes and of the changes in the small intestine have all helped to explain the difficulty in digesting food

Treatment remains difficult although the mortality has fallen to below 10 per cent in uninfected cases Deaths are usually due to collapse soon after admission or very serious infections, such as a fulminant pneumonia or tuberculosis The extreme anorexia the difficulty in digesting food, an intolerance to all fats and to many carbohydrates and sugars including glucose and lactose makes treatment difficult Skim milk powder or, in the most severe cases,

calcium caseinate (a more purified form of skim milk protein and one which contains little lactose), combined with a suitable form of carbohydrate, remains the treatment of choice and may initially be given by tube-feeding The principles and methods of treatment have been set forward recently by Dean (1952a) It is important to start treatment of the infant somewhat slowly with about 20 gm daily of protein, derived from about 60 gm of ordinary skim milk powder For calories nothing appears better than fully ripe bananas, which are well tolerated as in celiac disease After a few days or weeks depending largely on the character of the stools, the diet can be increased very slowly, until eventually children of two years are receiving about 5 gm of protein per kg of body weight (after loss of the edema) and over 100 calories per kg That is to say the diet has about 5 gm protein per 100 calories almost all the protein, at least at first, being derived from animal sources Dean (1952a) has reported cures with vegetable protein supplemented by vitamins, including B_{12}, but in advanced cases skim milk protein remains the most efficient form of treatment

Within a few days, a definite response occurs The general condition im [illegible] diuresis occurs [illegible] "enamel paint [illegible] ls improve but tend to remain bulky and contain undigested food, and diarrhea is apt to recur if much carbohydrate or fat is taken and after every successive addition to the diet The pancreatic enzymes increase, the serum albumin rises very quickly, the gamma globulins increase and tend to remain high for a very long period of time (Anderson & Altmann, 1951)

As assessed by serial liver biopsy, fat starts to disappear rather slowly from the center of the hepatic lobules, that is around the central vein, but even after 4 to 8 weeks of treatment, a certain amount of fat is often still visible at the periphery of the [illegible] liver extracts all exert a lipot[illegible] sav whether any of [illegible] 1949) It is doubtful if special supplements of methionine or choline have been demonstrated to accelerate this slow lipotrophic action of whole animal protein although, in Chile, Meneghello and Niemeyer (1950) considered that some acceleration occurred in infants given 5 gm of choline hydrochloride daily, in addition to 3 5 gm milk protein per kg of body weight

It is but natural that it should be desired to study the effect of other nutriments, such as individual amino acids and vitamin B_{12} but this is very difficult if not dangerous, in severe cases of kwashiorkor for, unless they receive skim milk protein they soon die It is, therefore, essential to take cases that are less critically ill prescribe a diet which will prevent further deterioration but which will maintain them in almost a stationary condition during a test period of 10 to 20 days and then to add the single nutriment, the effect of which it is desired to study In actual practice, this is difficult and dangerous Observations so far have not demonstrated that supplements of methionine aid recovery No other amino acid has been tried by more than one observer No clinical response to vitamin B_{12} has been unequivocably demonstrated.

The administration of this vitamin even parenterally does not improve the anemia which is usually normocytic but may be slightly macrocytic The bone marrow is probably very seldom megaloblastic as in pernicious anemia It is usually normoblastic or macronormoblastic The anemia does not respond to folic acid or to refined liver extracts but it sometimes improves slowly while receiving iron Anemia is seldom severe unless some infection is also present It is probable that the anemia is due to protein deficiency and resembles that present in cases of massive albuminuria

A marked feature of many cases of severe kwashiorkor is that progress is almost always slow and many months often elapse before the child is normal in every respect No reason is known which will explain the very slow gain of weight usually encountered during the later months of treatment In this respect the recovery of cases of kwashiorkor appears fundamentally different from that seen in children of the same age who are recovering from a period of



Figure 7 Convalescent kwashiorkor showing pot belly thin limbs and pale ha

undernutrition and calorie deficiency Appetite is apt to remain indiffi and diarrhea is easily provoked in all cases of kwashiorkor The failur gain weight rapidly has not so far been explained by any infection, or irreversible change in the pathology or biochemistry Probably more a tion should be paid to the intake, the digestion and utilization of food, since probable that the first organ to suffer and possibly the last to recover is gastrointestinal tract The pancreas and liver are possibly affected later this sequence reflects the high protein requirements of these organs Clinic these convalescent children have swollen abdomens and thin limbs (FIGUR as two American doctors noted (Payne & Payne, 1927) when they desci kwashiorkor in the New World, but unfortunately for them, they suggi no satisfactory title and called the complaint "Edema disease of Haiti"

In conclusion, I should like to state that, if I were asked what clinical are of the greatest significance in kwashiorkor, I would answer, without he tion, "Appetite and interest" In the early decrease of appetite, whi almost abolished in all cases of severe kwashiorkor, this disease differs fu mentally from undernutrition on diets, normal in the proportion of the va nutriments, but reduced in the total amount In kwashiorkor, there is desire for food and little interest in life These are the stigmata of kwashio Is this a response to the fatty liver or, as I suspect, due to some change in functions of the hypothalamus, together with its numerous nervous connec and subject to various forms of chemical control? Why should appetite crease possibly at an early stage on diets having a low protein calorie r How does skim milk powder stimulate hunger in a child dying of kwashior Eventually, it stimulates interest in life and, when a child with kwashic smiles, he is well on the way to recovery

Summary

In recent years a disease process has been described in young infants common in tropical countries and whenever the diet of a young child con a low protein calorie ratio especially if the proportion of animal protein is and that of carbohydrate is high The disease is considered to be identic Mehlnährschaden and many cases have been described as "nutritional edei The latter term describes two or more disease processes firstly, undernutr terminating as hunger edema in which the diet although bal ed in its portions is small in amount and deficient mainly in ca secor kwashiorkor produced usually by a protein high diet, in amount adequate to satisfy ap ly in the la es n fat accumulate at the periphery c lobule and t to c parts of the lobule The liver m ut often in Kwashiorkor has a characterist l biochem recovery

The clinical picture cases of or in chil This is very characteristic althoug s, vari deficiency may appear At an early , it is organs whi the highest pro affec

atrophic changes and loss of secretions by the small intestine pancreas and fatty changes in the liver

These features all respond to protein rich diets especially milk protein and animal protein, and it is doubtful if any specific response has been demonstrated to methionine, choline vitamin B_{12}, or folic acid There is uncertainty concerning the mechanisms and the factors which are involved Animal protein especially milk is still the best treatment of advanced cases but prevention and the cure of the more numerous mild cases must lie in an increased intake of vegetable protein fortified possibly by factors unidentified at present

Apathy and anorexia are the stigmata of kwashiorkor and separate this disease process from that seen in undernutrition on a diet normal in its proportions but reduced in its amount, thus producing a disease process in which caloric deficiency dominates the response of the body

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VITAMIN E AND CAROTENOIDS IN THE BLOOD PLASMA IN KWASHIORKOR

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Deficiency of protein has been generally accepted as one of the main factors in the development of kwashiorkor Other dietary deficiencies which might aggravate the want of protein therefore deserve attention Experiments with animals have clearly demonstrated that their ability to subsist on diets low in protein is influenced by the allowance of vitamin E Thus Dam[1] found that young rats which were given a diet grossly deficient in protein survived longer when they were dosed with vitamin E than when they were left undosed Various other workers confirmed the beneficial effects of vitamin E in rats given diets low in protein both upon the body weight and in preventing dental abnormalities[2, 3, 4, 5] In view of the severe injury to the liver in kwashiorkor special interest may be attached to the finding of Schwarz[6] and others[7, 8] that vitamin E protects rats against the hepatic necrosis which is otherwise induced by protein deficiency

It seemed desirable, therefore, to try to obtain evidence on the vitamin E status of patients with kwashiorkor by estimations made on the blood plasma Specimens were sent by air from Uganda to Cambridge, mostly in 1950 but a few in 1951 The plasma was shaken up with ethanol and light petroleum and total carotenoids were estimated by their yellow color in the extract separated after centrifuging The extract was then evaporated, and the residue was redissolved in hot ethanol Cholesterol and other sparingly soluble substances were removed by freezing and again centrifuging and "total reducing substances" were estimated in the supernatant solution with *αα'* dipyridyl and ferric chloride 'vitamin E' was measured as the difference between the total reducing substances and the correction appropriate for the carotenoid For purposes of comparison, estimations were also made upon specimens of plasma from healthy male African adults and from a few healthy infants Specimens were also taken from adults suffering from pyomyositis, which is a disease characterized by degeneration of the voluntary muscles and the formation of abscesses

From TABLE 1 it will be seen that lower levels were found for vitamin E in kwashiorkor (average 0 67 mg per 100 ml) than in healthy men (average 0 92 … The reduction of carotenoids … 100 ml as compared with … more noticeable Several of the specimens of plasma gave extracts w… red quite colorless to the eye The levels in pyo… sitis also fell be… lues for healthy subjects both in vitamin E … noids

From these resul… ppear either … ake of vitamin E and carotenoids is low … r or that t… ir efficient absorption from the … carriage by … idence of

TABLE 1

	No. of cases	Av age	Carotenoids µg 100 ml.		Tocopherol mgm. 1000 ml.	
			Range	Av	Range	Av
Kwashiorkor infants	12	2 [illegible]	4 54	23	0 35–1 06	0 67
Normal African infants	4	2 [illegible]	46–142	107	0 85–1 40	1 10
Normal African adult males	10	[illegible]	49 167	99	0 58–1 37	0 92
Pyomyositis in adults	17	[illegible]	13 89	38	0 34 1 38	0 80

low values for the vitamin E has already been found by Darby *et al*[9] in pa

disease, cirrhosis of the liver, sprue, celiac disease, and diarrhea All patients with these diseases had their lowest readings in repeated observations within the range of 0 06–0 25 mgm The values found in kwashiorkor, therefore, may seem no lower than might be expected in a disease which is characterized by damage to the liver and general impairment of the absorption of food It is perhaps surprising that the ranges observed did not differ more widely from those found in pyomyositis a disease in which injuries to the liver are less prominent than in kwashiorkor, but in which a suspicion of vitamin E deficiency might possibly be suspected from the muscular degeneration Presumably the frequency of fever influenced the levels in this disease

Even if we regard the reductions in vitamin E and carotene mainly as sec

servations in a case of sprue It cannot be ruled out, therefore, that a secondary deficiency of vitamin E may be included among the numerous complications and aggravations which are involved in the progress of kwashiorkor Our very low values for carotene suggest further that the vitamin A status may be endangered when the reserves of the preformed vitamin which are presumably stored during lactation have been used up

Summary

Low values for vitamin E and carotenoids, probably related to impaired powers of absorption, were found in the blood plasma of African infants suffering from kwashiorkor

The possibility of the occurrence of secondary deficiencies of subnormalities of vitamins E and A as complications to kwashiorkor should not be overlooked

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ASPECTS OF NUTRITIONAL LIVER DISEASE—HUMAN AND EXPERIMENTAL

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In this communication we propose, first to examine the relationships of hepatic fibrosis to antecedent fatty change and to necrosis of the liver in malnourished human beings and in animals fed various diets and, secondly, to [illegible]

The Fatty Liver and Hepatic Fibrosis

A fatty change can be found in livers of malnourished infants and children admitted to hospital during an acute nutritional breakdown (Normet, 1937, Bablet and Normet, 1937, Gillman *et al*, 1945, 1951, Waterlow, 1948, Van der Sar, 1951, Oomen, 1951, Altmann, 1948) By serial biopsy examination, we [illegible]

Six monthly biopsies of the livers of infants and children who have left hospital after recovery from an acute nutritional breakdown show no evidence [illegible]

infant and child pellagrins admitted to hospital, we arrived at the conclusion that although fatty livers may be associated with many diseases fatty change is more frequent and is likely to be more severe in African infants admitted to hospital with nutritional edema or infantile pellagra than in children of the general population

The fatty liver in infants suffering from nutritional edema and pellagra is therefore of relatively acute onset and in a sense is similar to the intensely fatty livers observed in well nourished children dying four to eight days after an acute attack of "summer diarrhea" or after severe burns Like the other events associated with the acute episode, a fatty liver is an expression of a relatively sudden and profound disorder of metabolism occurring in a malnourished infant (Gillman and Gillman, 1951)

One question now arises whether the fatty liver in any way influences the development of fibrosis At the outset, we may say that portal fibrosis, or more than usual amounts of fibrous tissue, in the liver occur frequently in

adult Africans (Gillman and Gillman, 1951) It now remains to attempt a correlation between the frequency of hepatic fibrosis and steatosis

Twenty five of 261 African adults (*i e*, 9 5 per cent) dying accidentall showed gross portal fibrosis as compared with 11 9 per cent of 177 adult African pellagrins admitted to hospital In the general population the incidence of the fatty liver in Africans dying from accidental causes was 2 7 per cent

In the livers of 79 adult females over the age of twenty years admitted to hospital during an attack of pellagra, 66 (86 per cent) contained some stainable fat and, of these, the livers were moderately or intensely fatty in 35 females (4 per cent) On the other hand, some fat was detected in the livers of 65 (69 per cent) of 94 adult male pellagrins in our series and, of these livers 33 (3 per cent) contained moderate or abundant amounts of fat Apart from demon strating the greater incidence of fatty change in females this analysis reveals that a fatty liver occurs much more frequently in pellagrins than in members of the general population

We are led to conclude that there is no *prima facie* case for claiming th existence of a direct causal relation between the fatty liver and hepatic fibrosis in the malnourished African The evidence adduced in this connection is a follows (1) The fatty change in the livers of infant and adult Africans is of relatively acute onset, (2) After the acute episode the fat usually disappears from the liver, (3) Sampling of the livers of the general population at all ages affords no evidence of a persistent and progressive fatty change, (4) The evidence of hepatic fibrosis in the general population of Africans cannot be correlated positively with the frequency of hepatic steatosis Taking this evidence into account, we affirm once more that the fatty liver, *per se* in the malnourished infant and adult African suffering an acute nutritional breakdown plays no direct role in promoting the high incidence of liver disease in Johannesburg Subsequent to our own study Dible (1951) independently arrived at the conclusion that even in London there was little evidence to show that fatty infiltration of the liver was a precursor of hepatic fibrosis But it has been shown by Dible (1951) and by Gillman and Gillman (1951) that a fibrotic liver can undergo a fatty change in which case it might easily be possible to mistake the sequence of these two reactions and to regard the fatty change as precedin the fibrosis

The frequent coexistence of fat and fibrosis in the same liver has led to the generally held opinion that long standing fatty change in the livers of animal on various diets is an essential precursor of diffuse hepatic fibrosis (Himsworth 1947) This view implies that the fat filled enlarged hepatic cell occludes the circulation through the lobule and eventually results in necrosis of the paren chyma Necrosis in turn is said to stimulate the connective tissue reaction an essential precursor of hepatic fibrosis It would thus appear that, in the fatty liver as in the necrotic liver to be mentioned below, necrosis is the precursor of hepatic fibrosis

From our own observations in animals we have demonstrated that certain diets will favor the development of an extensive fatty change which can persist for approximately 300 days without any evidence of fibrosis (Gillman *et al* 1953) Since it is possible to disengage the fatty change from fibrosis it fol

lows that the accumulation of fat and the overgrowth of fibrous tissue in th
-eparate processes which are not necessarily causall
ibrosis can be disengaged from steatosis Eac
dently of the other, and each requires special cor
ditions if the appropriate reaction is to be set into operation Nutrition
experiments in animals have shown that as a rule, fibrosis only supervenes in
fatty liver if the latter is progressive and long standing Such a relationshi
we have shown does not exist in malnourished Africans Although nutritio
plays a commanding role in promoting the frequency of fibrosis in the ma
nourished African it does so in other ways than by exciting an anteceden
intense fatty reaction

We wish to point out that the fatty change in the liver may participate ir
directly in the development of hepatic fibrosis An intensely fatty liver i
pellagrins is indicative of an acute metabolic disorder On each occasion whe
the liver undergoes a fatty change, there is a katabolic process of greater c
lesser severity which has as one of its consequences, irritation of the reticul
endothelial system Repeated irritation of the reticulo-endothelial syster
over many years often beginning in infancy, can, in some circumstance
culminate in portal fibrosis

Necrosis and Hepatic Fibrosis

Acute necrosis of the liver has not been established in infant and adu
Africans admitted during an acute nutritional breakdown It is true th

ing to autopsy We would like to mention that the administration of thiami
to one of our pellagrins unquestionably precipitated diffuse necrosis of th
liver recognized from fragments obtained by puncture biopsy (Gillman an
Gillman 1951)

There are other circumstances however, usually those associated with acut
anoxia which may contribute to necrosis of the liver (Gillman and Gillma
1948) In the first place the frequent use of open braziers to heat ill ventilat
huts and shacks during the winter months leads frequently to carbon monoxi
poisoning In a small series of 25 cases of fatal carbon monoxide poisonin
we observed hepatic necrosis in two cases surviving for longer than nine hour
Secondly severe hemorrhage resulting from industrial or other accidents an
from assaults during drunken brawls produces severe anoxic reactions in t
liver If such individuals survive for a few days the anoxic reaction ma
eventually culminate in necrosis We reported two cases of extensive necros
of the liver following severe hemorrhage in forty cases of multiple injurie
It must be remembered that many of the cases died within a few hours of th
injury but they did show an intense reaction in the form of vacuolated an
plantlike cells which as we have shown can go on to necrosis Similar plan
like cells apparently precede the necrosis of infective hepatitis as shown e
perimentally by Kyo Hara *et al* (1952)

Thirdly, severe infections especially those associated with lobar and bronch

pneumonia, often precipitate jaundice in the African In some of these cases coming to autopsy, we have found necrosis of the liver Fourthly, prior to the introduction of antibiotics, intensive and often indiscriminate use of arsenic, ostensibly for the treatment of syphilis among the Africans, may also contribute to the production of hepatic necrosis We mention this because of the numerous severe reactions we have observed in Africans receiving arsenic, who were sufficiently indisposed to be admitted to hospital But not all patients, even though indisposed seek hospital treatment after arsenic therapy The untoward reactions experienced are often regarded as part of the treatment Fifthly, the frequency of tuberculosis among the Africans in Johannesburg and elsewhere in this country is so great that it is regarded as a major health problem Extensive tuberculosis of the liver is a common finding Even when less extensive, we have found more than suggestive indication that tuberculosis can contribute to a low grade but widespread destruction of liver tissue

In this connection, we should like to mention that Straub and Schaberg (1950) have drawn attention to the frequency of hepatitis, allegedly due to a virus, in malnourished Indonesians Perusal of their protocols disclosed that, in every instance cited, a large or small focus of tuberculosis in the lung was noted Straub and Schaberg regard the prevalence of hepatic fibrosis in Indonesia as an interaction of two factors, namely, malnutrition and virus infection While we are not entirely satisfied that Straub and Schaberg have made out a case for virus infection in the Indonesian, nevertheless, in view of the greater vulnerability of the liver to infection in malnourished individuals it seems to us that the role of the tubercle bacillus, as an etiological agent in the production of hepatic necrosis and fibrosis in malnourished individuals, has not been adequately assessed Moreover, it is our opinion that not only virus infection and tuberculosis but also parasitic infections, such as schistosomiasis and malaria, as well as other infections, including those responsible for lobar and broncho pneumonia, may contribute collectively, either directly by destruction of liver tissue, or indirectly through the production of severe anoxia, to the greater incidence of necrosis of the liver in a chronically malnourished population

Although we have seen cases with acute and subacute yellow atrophy of the liver in Africans, it is impossible, in the absence of further laboratory examinations, to incriminate the virus of infective hepatitis Besides the use of medicinal herbs by Africans has been repeatedly suspected of promoting extensive necrosis of the liver of the type mentioned above

As in the case of the fatty change necrosis has been regarded as a precursor of hepatic fibrosis However, it is important to realize that fibrosis is not an automatic response of the liver to necrosis Necrosis can be disengaged from fibrosis Both in man and in animals it has been repeatedly demonstrated that, even after extensive injury to the liver, regeneration may be complete [Dible *et al* (1943) Lucké (1944), Cameron and Karunaratne (1936), Orr (1940), Gillman *et al* (1952 b)] On the other hand, we have demonstrated in animals that whether or not hepatic fibrosis will supervene in a liver which has undergone extensive necrosis of dietary origin is not only a function of

necrosis but also of the diet If, after necrosis has declared itself, the animals are still maintained on the deficient diet, recurrent attacks of necrosis are to be expected In this context, provided the animals survive for any length of time, hepatic fibrosis and even nodular hyperplasia are almost inevitable consequences On the other hand, if necrosis has declared itself and the animals are then given access to a balanced ration, regeneration of the liver is usually complete and fibrosis is prevented Moreover, by allowing the animals access alternately, for varying periods to the necrogenic and to the balanced diet, it is possible to produce a variety of pathological reactions in the liver These can be interpreted as representing a combination of necrosis, regeneration, and fibrosis Gross distortion of liver structure can be one of the end pictures (Gillman *et al* 1952, a and b)

It is evident from the latter experiment that, although survival of animals fed a necrogenic diet can be prolonged by occasional feeding for limited periods, of a balanced ration, the liver still remains vulnerable and can still be grossly disorganised on a later occasion Clearly, the more chronic forms of liver

to differences in the ability of rats to take advantage of the good diet, which is, in turn, determined by the extent of pre existing liver and other organ damage imposed by the necrogenic diet

to regeneration in better fed animals Diet then may serve to increase the frequency of hepatic fibrosis in two ways firstly, by rendering the liver more vulnerable to noxious agents and especially to the effects of acute anoxia, and secondly by promoting fibrosis once necrosis has supervened

One question still remains unanswered Why is there such variability in liver pathology in different members of the same malnourished population? The answer may be given, in part, in terms of the subtle differences in diet consumed by members of the population, the extent to which each individual may gain access to better food during times of plenty, the presence or absence of various infections including pneumonia, tuberculosis, malaria schistosomiasis *etc*, and the extent to which occupation and living conditions expose individuals to severe liver injury

In addition, the difference in the reactivity of livers of malnourished individuals probably has the same basis as the differences observed in the liver of seemingly well nourished patients contracting infective hepatitis during the same epidemic. Of those suffering from infective hepatitis, some may suffer a mild attack and recover rapidly, others develop hepatic necrosis which heals completely, whereas in still others the necrosis may go on to fibrosis. In these instances, the virus of infective hepatitis merely served to expose underlying differences in metabolism of affected individuals already existing even before the disease was contracted. That is to say, although cases contracting infective hepatitis but reacting differently to the virus had ready access to a good diet and were not showing signs of malnutrition, such individuals were probably utilizing their food in different ways. After all, it is well known that two individuals, consuming similar diets, may put the nutrients to different uses. One individual may increase rapidly in weight and another may remain thin.

In this connection, we may recall the classical experiments of Chaikoff and his associates (Chaikoff *et al.* 1938, 1948, Gillman and Chaikoff, 1949) who demonstrated the altered utilization of food in thyroidectomized, hypophysectomized and pancreatectomized dogs. Fatty livers developed in all these animals while consuming a diet rich in lipotropes and adequate to prevent a fatty liver when such diet was fed to normal dogs. However, the fatty liver of the dogs with one or other of the endocrine glands ablated could be prevented by increasing the choline intake in amounts which ordinarily would not be available in an average diet.

By analogy, human subjects suffering from varying degrees of subclinical hypothyroidism or of pancreatic disease will not utilize the same food in the same way as another individual with a normally functioning thyroid or pancreas. Indeed, as revealed in the case of hypothyroidism, nutritional disease may even supervene on a diet adequate to maintain an intact organism in good health (Gillman and Gillman, 1951). If then, two individuals, one in good health, the other suffering from subclinical hypothyroidism, are both consuming the same diet, the diet will in the one case be adequate and in the other, deficient. If these two individuals both develop an attack of infective hepatitis divergences in hepatic reactions are to be expected in the same way as has been shown in the case of the reaction of livers of animals subsisting on different diets and poisoned with the same concentration of chloroform. The divergent reaction of livers to the same stimuli, therefore, is dependent on the prevailing metabolism. The latter is not only a function of the diet consumed, but also of the way the nutrients are utilized. The utilization of food in turn is dependent on the functional integrity of the various organs and tissues and the way these various components of the organism are functionally related (Gillman and Gillman 1951). The difference in liver structure observed in a random sample of a malnourished population, therefore, can be explained on the same basis as mentioned above in the case of infective hepatitis.

In addition however consideration needs to be given to the number of previous nutritional breakdowns the age at which the first breakdown occurred, the extent of collateral organ damage precipitated by infections, *etc.*, as well

as to the particular economic circumstances of each individual from birth until the time the liver is examined

The search for an understanding of the mechanism underlying the prevalence of hepatic disease and more especially of hepatic fibrosis in a malnourished population demands a wider appreciation of the complex factors involved in a biological reaction As will be indicated in the final section of this paper, the approach to the problem of nutritional disease is not to be sought only in terms of single factors

Methodological Considerations of Nutritional Investigations

It has become common practice among experimentalists to design diets in such a way that the presence or absence of a specific factor may decide whether or not spontaneous liver disease will emerge, or whether or not the liver will be protected against toxic agents As a consequence, they have identified

With the exception of cystine, the general trend has been toward accepting liver disease as the consequence of a lack of one or other constituents in the diet The inclusion of 1 per cent cystine in the diet can lead to extensive hepatic necrosis and hemorrhage within a few days (Earle and Victor, 1941) In this instance, a causal relationship can be established between the contents of the diet and the onset of liver necrosis Even in those diets where the absence of a particular factor can be shown to be related to a particular pattern of liver reaction, too little consideration has been given to defining the type of metabolism promoted by the diet which in the first instance allowed the particular factor to be all important in determining whether or not the integrity of the liver would be maintained If the emphasis is placed mainly on the

of choline and cystine (Glynn *et al*, 1945), or alpha tocopherol (Himsworth and Lindan 1949, Gyorgy *et al* 1950) or by antibiotics (Gyorgy, 1951), (2) Necrosis of the liver in rats fed on the low protein diet used by Hove *et al* (1949) may be prevented by tocopherol but not by methionine, (3) Necrosis of the liver in rats fed potato starch and food yeast (low in proteins) may be prevented by alpha tocopherol or methionine, but not by cystine (Gillman *et al* 1952a) (4) Diffuse necrosis can occur even on high protein diets lacking vitamin A and fat but containing alpha tocopherol and can be prevented and cured by the addition of vitamin A (Gillman *et al* 1952a)

It is quite clear that, in these different circumstances the constituents of the diet rather than the intrinsic "needs" of the organism have been collectively responsible for imposing a metabolism which required the further inclusion of

a specific factor in the diet for the prevention of liver disease Moreover we have demonstrated that in so-called low protein diets factors other than protein can decrease the incidence of hepatic necrosis Himsworth (194 wrote The most discordant results were received and it is no exaggera or to say that at one time or another every dietary component save carbohydrat has been indicted (as the cause of liver injury)' We have since shown tha even the nature of the carbohydrate can modify the incidence of necros (Gillman *et al* 1952a)

Clearly then the constituents of the diet play a commanding role in crea n the conditions for making a specific nutrient a limiting factor in a nutr t on syndrome If the significance of a particular nutrient is to be assessed it equally important that the conditions be established which allow the nutri to occupy such a commanding role in preventing or promoting a disease proce of nutritional origin In support of this opinion we should like to prese some of our own observations on the prevention or the facilitation of the fat liver in rats

Using maize as the main constituent of the diet a fatty liver is much mo easily produced in rats than is hepatic necrosis (TABLE 1) If maize alone fed to adult rats (TABLE 1, Diet 1), in the absence of any intercurrent infecti or interference with food intake consequent on injury to the teeth a fa change commencing as early as the sixteenth day of the experiment decla itself in

liver

may be

maize a

tion of other nutrients however it is possible even in young rats to p o consistently a fatty change in the liver (TABLE 1 Diets 7, 8 10 and 11) T most intensely fatty livers were found in young animals fed Diets 10 and and the least intensely fatty in rats fed Diet 7 If the salt mixture is omit (Diet 9) the fatty change does not appear in the liver Apart from congest no other gross or microscopically demonstrable pathology is evident

It might be argued according to the views expressed by Handler and Du (1946) that the absence of the fatty liver in young rats is due to the failu of these animals to grow to any extent We have observed fatty livers in r fed 10 per cent brewers yeast containing an equivalent of 0 5 per cent adc cystine and potato starch even though the growth was scarcely different fr that of young rats which failed to develop fatty livers while subsisting on ma alone Then too we have produced a fatty change in the livers of you rats on a diet consisting of 10 per cent vitamin free casein 30 per cent corn 50 per cent sucrose 4 per cent agar 4 per cent salt mixture, and 2 per c cystine together with an appropriate vitamin mixture The growth of th animals was of the same order as that of rats fed Diet 9 in which the salt m ture was absent In the latter animals (that is those on Diet 9) neither fatty liver nor hepatic necrosis was observed Clearly then neither the fa change nor necrosis of the liver is necessarily a function of a minimum amo of growth in the conditions of our experiment

The quality and quantity of proteins in the diet are generally believed

TABLE 1

Diet no.	Maize	Glucose	Food yeast	Casein	Skimmed milk powder	Salts	Fat	Vitamins A, E, & D	Protein content of diet & (per cent)†	No. of rats	Average initial weight (gm.)	Average maximum weight (gm.)	Period of survival (days)	State of the liver
1	100	–	–	–	–	–	–	–	8.1	30	261	244	350–400	Intensely fatty.
2	100	–	–	–	–	–	–	–	8.1	15	34	40	65–100	Atrophic
3	90	–	10	–	–	–	–	–	12.2	5	40	64	58–69	Atrophic
4	95	–	–	–	5	–	–	–	9.1	5	34	85	142–231	No fatty change; focal necrosis.
5	80	–	–	20	–	–	–	–	26.5	5	35	68	62	Congested
6	97	–	–	–	–	3	–	–	7.8	5	32	75	104–163	Mildly fatty.
													184*	
7	72	–	10	5	–	3	10	–	15.7	5	40	340	391‡	Mildly fatty
8	82	–	10	5	–	3	–	+	16.5	5	29	315	234*	All moderately fatty
9	75	–	10	5	–	–	10	+	15.9	5	29	88	65–125	Congested
10	77	–	10	–	–	3	10	+	11.1	5	34	254	238*	Intensely fatty
11	92	–	–	5	–	3	10	+	11.6	5	32	206	244*	Intensely fatty
12	82	–	–	–	5	3	10	+	8.2	5	30	206	255*	Moderately fatty
13	67	–	–	–	20	3	10	+	11.6	5	31	373	242*	No obvious pathology
14	–	67	–	–	20	3	10	+	6.2	5	28	121	255*	No obvious pathology.
15	57	–	10	20	–	3	10	+	29.5	5	31	325	234*	No obvious pathology
16	72	–	10	–	5	3	10	+	12.3	5	39	305	254*	4 intensely fatty

* Represents the day on which *all* rats in the experiment were sacrificed.
† Protein determined as nitrogen.
‡ Rats still alive, liver biopsy at 391 days.

determine whether or not fat will accumulate in the livers of experimen

that our own investigations reveal the unsettled role of proteins in promoting or preventing the fatty liver

From TABLE 1, it is evident that the fatty liver develops in young rats fe Diet 7 and Diet 11 In heavy weight (adult) rats, maize alone is efficient as fatty liver producing diet If we examine the protein content of all these di (TABLE 1), it is clear that in our experiments the fatty liver was produced wi protein concentrations ranging from 8 1 per cent to 15 per cent In the ca of the maize alone (Diet 1) the fatty liver could be prevented or cured by th administration of 50 mg of methionine per rat per day This latter observa tion would seem to indicate that, even in diets with over 15 per cent prote there is still a lack of methionine or of proteins containing an equivalent that amino acid Further confirmation would seem to be available from t fact that by increasing the casein from 5 per cent to 20 per cent so that t total amounts of proteins reached 29 per cent (Diet 15), the fatty liver w prevented

When, however, maize and 5 per cent dried skimmed milk were fed to r (yielding an over all protein content of 9 1 per cent), the liver did not show a fatty changes (TABLE 1 Diet 4) Similarly, when a diet containing gluc and 20 per cent skimmed milk powder (TABLE 1 Diet 14) yielding a total 6 20 per cent protein was fed to young rats, the fatty liver was again p vented It might be argued that 6 2 per cent of dried skimmed milk prot provided sufficient methionine to prevent the fatty liver On the other ha 5 per cent dried skimmed milk in the maize diet (Diet 16) contains about third the protein in 5 per cent casein The latter diet favored the accumula of fat and the former did not Five per cent casein contains more methion than does 5 per cent dried skimmed milk Yet the dried skimmed milk is m effective in preventing the fatty liver induced by a predominantly maize d It should not be forgotten however that, whereas casein is a pure prote dried skimmed milk contains in addition to some proteins, other fact which constitute approximately 69 per cent of the dried skimmed milk Th other factors were apparently as important as the small amount of prot in preventing hepatic damage It is imperative to stress the fact that dr skimmed milk or even whole milk cannot be regarded as consisting only proteins however efficient dried skimmed milk may be in treating malnours infants in Uganda [Dean 1952 Trowell *et al* (1952), Editorial (British M J, 1952)] Since we have already indicated that the omission of salts fro maize diet can prevent the fatty liver (compare Diets 9 and 10) it follows t explanations of the complex chemical processes underlying the productior the fatty liver in terms of single factors are untenable

From these seemingly paradoxical observations it is evident that as in
...trients ir
The
setting

constellation promoting hepatic steatosis (compare Diets 10 and 11 with Diets

pected in nutritional investigations Thus, the addition of 10 per cent food yeast to potato starch results in 95 per cent incidence of hepatic necrosis in young rats, whereas 10 per cent food yeast and 90 per cent maize meal is invariably associated with severe deformity of the bones Obviously, the food yeast maize combination provokes the pathological changes in the rats by virtue

By devoting exclusive attention to the deficiencies of maize, the peculiar properties of the constituents of maize are quite naturally overlooked It is common knowledge that zein, the main protein of maize, contains hardly any lysine and practically no tryptophane Proteins which contain considerable

published information, the unbalanced *amino acid content* of maize is less well appreciated Six amino acids namely glutamic acid, tyrosine, phenylalanine, proline, leucine, and isoleucine account for 78 per cent of the total amino acids in zein In this connection, too, it might be added that 62 per cent of the amino acids of gliadin of wheat are made up of glutamic acid, proline, leucine, and isoleucine. This disproportionate concentration of some amino acids can have serious consequences for the organism and, undoubtedly, can acquire *toxic properties in some dietary settings.* Several investigators have disclosed that, in appropriate circumstances, feeding excessive amounts of one amino acid can excite severe toxic reactions in experimental animals One need only refer to the acute necrosis of the pancreas following the exhibition of excess tyrosine (Hueper and Martin, 1943) However, even in physiological concentrations, *an amino acid such as cystine may be required in some dietary settings to produce* hemorrhagic nephritis or acute necrosis of the liver (Griffiths and Wade, 1939) Moreover, it has been shown that an excess of one amino acid, such as glycine calls for an increase in the pteroylglutamic acid in the diet if the growth inhibiting effect of glycine is to be prevented (Dinning *et al*, 1949) That is to say, *an abnormally high concentration of an amino acid sharpens* the need for particular vitamins and other food factors in amounts which are out of proportion to the requirements of the organism subsisting on a different diet

determine whether or not fat will accumulate in the livers of animals The numerous reports published during the last decade afford support for this belief Without wishing to reopen the entire problem of relation of proteins to the fatty liver, we should like to mention at this sta that our own investigations reveal the unsettled role of proteins in or preventing the fatty liver

From TABLE 1, it is evident that the fatty liver develops in young rats f Diet 7 and Diet 11 In heavy weight (adult) rats, maize alone is efficient a fatty liver producing diet If we examine the protein content of all these d (TABLE 1), it is clear that in our experiments the fatty liver was produced w protein concentrations ranging from 8 1 per cent to 15 per cent In the c of the maize alone (Diet 1), the fatty liver could be prevented or cured by administration of 50 mg of methionine per rat per day This latter obser tion would seem to indicate that, even in diets with over 15 per cent prot there is still a lack of methionine or of proteins containing an equivalen that amino acid Further confirmation would seem to be available from fact that, by increasing the casein from 5 per cent to 20 per cent so that total amounts of proteins reached 29 per cent (Diet 15), the fatty liver prevented

When, however, maize and 5 per cent dried skimmed milk were fed to (yielding an over all protein content of 9 1 per cent), the liver did not show fatty changes (TABLE 1, Diet 4) Similarly, when a diet containing gl and 20 per cent skimmed milk powder (TABLE 1, Diet 14) yielding a tot 6 20 per cent protein was fed to young rats, the fatty liver was again vented It might be argued that 6 2 per cent of dried skimmed milk pro provided sufficient methionine to prevent the fatty liver On the other h 5 per cent dried skimmed milk in the maize diet (Diet 16) contains about third the protein in 5 per cent casein The latter diet favored the accumula of fat and the former did not Five per cent casein contains more methio than does 5 per cent dried skimmed milk Yet the dried skimmed milk is effective in preventing the fatty liver induced by a predominantly maize It should not be forgotten however that, whereas casein is a pure pro dried skimmed milk contains in addition to some proteins other fa which constitute approximately 69 per cent of the dried skimmed milk T other factors were apparently as important as the small amount of pr in preventing hepatic damage It is imperative to stress the fact that skimmed milk or even whole milk cannot be regarded as consisting on proteins however efficient dried skimmed milk may be in treating malnour infants in Uganda [Dean 1952 Trowell *et al* (1952), *Editorial (British J*, 1952)] Since we have already indicated that the omission of salts fr maize diet can prevent the fatty liver (compare Diets 9 and 10), it follows explanations of the complex chemical processes underlying the productio the fatty liver in terms of single factors are untenable

From these seemingly paradoxical observations it is evident that as in case of the production of hepatic necrosis a minimum number of nutrients be present in the diet to promote the accumulation of fat in the liver Th ture of the metabolism stimulated by these nutrients provides the settin

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In searching for proteins to supplement maize it is not only desirable to find protein containing sufficient amounts of lysine and tryptophane but care should also be taken not to increase still further the already high level of glutamic acid known to be present in maize Our experimental studies have led us to assert repeatedly that, although a minimum number of food factors is required by the organism to promote growth and differentiation the precise requirements of the organism for any particular food factor are a function of the quality and quantity of the other constituents of the diet

In the present state of knowledge, we may affirm that generalizations based *on particular experiments about the requirements of organisms* for particular food factors are not only unjustified but can be grossly misleading in the management of nutritional disease (Gillman and Gillman 1951) Such generalizations are all the more to be condemned when they are founded on short term experiments or merely on the growth curves and survival of the animals.

Equally unjustified are the assertions of Brock and Autret (1952) that dried skimmed milk is synonymous with protein As mentioned above approximately 30 per cent of dried skimmed milk consists of protein We ourselves have demonstrated that, whereas dried skimmed milk may assist in the rapid recovery of pellagrous infants, thus confirming the original observations of Waterlow (1948), the addition of an equivalent amount of casein had no such beneficial effect

The constellation of factors governing the integrity of the liver and of other tissues is now so great that any claim made for the efficiency of a particular factor in safeguarding the liver and other tissues can have only a limited application In approaching the problem of human malnutrition it is as well to remember the timely observations of Adolph (1943), made in connection with water metabolism "No sign from heaven is likely to point out one key that unlocks the elementary activities The theory that a single governor exists for a physiological activity does not appear now to be substantiated Moreover, there is no means of recognizing a regulator even if examined. A specific volley of nerve impulses or an isolatable extract are possible links in the chain or elements in a complex Repeatedly it is found that many factors vary simultaneously, each one is as central as any other and only by convenience of thought is one exalted above another"

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being must be accepted. When we use the word "activity" in this paper, we mean activity *in vitro* under stated conditions

In theory, there are several ways in which enzyme activity or concentration might be affected by dietary deficiency Since enzymes are proteins and, therefore, presumably in a state of rapid turnover, the rate of renewal of the enzyme molecule may be reduced if the supply of dietary protein is inadequate There is indeed, ample experimental evidence that deficiency of protein or of single amino acids can cause changes in the enzymatic pattern of the cell, some enzymes being reduced, other selectively preserved[8-11] Although no clear picture with physiological meaning has yet emerged from this work, it is reasonable to suppose that in man as in animals dietary deficiency may cause qualitative changes, if not actual disruption, in the enzymatic machinery of the cell

Another possible effect of malnutrition on enzymes is a kind of disuse atrophy Potter,[12] discussing the purpose and justification of enzyme assays, wrote "The determination of the amount of enzyme in a cell would have no value whatever, were it not for the fact that the amount of an enzyme is probably closely related to the extent of its use" He was considering the enzyme pattern in relation to cell growth and specialized function It is only a short step from this idea to the possibility of a loss of enzyme from lack of substrate, the converse of the adaptive enzyme formation that is known to occur in bacteria, and of which there seem to be some examples in mammalian tissues[13-16] If such a process does occur, the effects of protein deficiency should be most apparent in those tissues which are most active in the formation or metabolism of protein It is perhaps, more than a coincidence that in kwashiorkor where protein deficiency plays a prominent part the clinical and pathological evidence shows that the organs most affected are those which are known on physiological grounds and from isotope work to have the highest protein turnover namely pancreas liver and gut It is clear that if the synthetic mechanisms are damaged, either from lack of components or from disuse, the condition must become progressively more difficult to reverse This may explain why such a long time is sometimes needed to restore the malnourished body to normal Not only do the building blocks have to be supplied, but the synthetic machinery must itself be rebuilt

Finally enzyme activity may also be affected by shortage of essential co-factors[17, 18] This point needs no elaboration In this connection however, the work of Jacquot[19] re-emphasizes the difficulty of making deductions about the behavior of the whole animal from the results of experiments *in vitro* He has shown that, even in fatal pyridoxin or biotin deficiency there is no change in over all nitrogen metabolism or in the output of urea in the urine

These are some of the speculations underlying this work It is clear that even if changes in enzyme activity are found their interpretation will not be easy

Material and Methods

All this work has been done on infants between the ages of six months and two years. The plan of investigation was simple As soon as possible after

ENZYME ACTIVITY IN FATTY LIVERS IN HUMAN INFANTS

By J C Waterlow and S J Patrick

Medical Research Council, London England, and Department of Physiology, University College of the West Indies, B W I

This paper is an account of work in progress, presented in the hope that it may give some clue to fruitful lines of attack in the future Moreover, since the work is in a provisional stage, we shall perhaps be forgiven if more attention than usual is given to objectives and methods, rather than to actual results

Our studies on the activity of enzymes in the human liver are based on the very simple idea that if, in man as in animals, structural damage to the liver can be caused by dietary means, that damage should be preceded by a metabolic change or biochemical lesion If a proper perspective is to be kept, how

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It may, therefore, be legitimate to regard liver injury not as a local p

but as a particular example of the effect of an adverse environment on glandul organs throughout the body This aspect has been very little studied in th rat Secondly, in the case of the liver, although the broad similarities a suggestive, there is not an exact correspondence between the types of inju produced experimentally in the rat and those found clinically in man. Fat liver in man differs from its analogue in the rat both in pathology and in respo to treatment In man, the fat is peripheral,[1] in the rat, central[2] This d ference is surely not without significance In man, a high protein diet a particularly milk, seems to be more effective in removing fat than either choli or methionine[3] Again although human diets in many parts of the wo are low in sulphur amino acids, there is no good evidence that massive necro of purely dietary origin occurs in man On the other hand, some types liver damage in association with malnutrition or a low protein intake which as yet there is no experimental analogue, have been described in m These include pigment cirrhosis in South Africa[4] infantile cirrhosis in Ind and serous hepatosis in Jamaica[6] For these reasons we must be cautious applying to man the results of animal experiments, a conclusion that is su ported by some of the biochemical data reported in this paper

Few people however would now deny that there is such a thing as nu tional liver injury in man In the search for what the Gillmans[7] have cal the "altered metabolic regulations" underlying the structural changes seemed best to concentrate on the dynamic aspects rather than to meas the levels or concentrations of metabolites in the tissue The study of zyme activity promised to be a useful tool, more sensitive than the classi liver function tests and going nearer to the root of the problem There m well be, of course a great difference between the activity of an enzyme measu *in vitro* under standardized and artificial conditions and the activity in natural environment in the body In the former case, we are measuring w it can be made to do not what it actually does This limitation for the t

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so that clearly our sample may not be representative We have not felt it justifiable to try and get more than one specimen at any given time The best, therefore, that can be done to solve this difficulty is to take two separate fragments from the same specimen (when enough tissue is available) and make [illegible]

[illegible]

from one lobule to another rather than from one large area of the liver to another In general, such comparisons have shown good agreement between duplicate homogenates[22]

The second difficulty is the basis of representation. Enzyme activity is usually expressed per milligram dry weight of tissue In our material, dry weight must clearly be corrected for fat, and probably for glycogen also, but even when this is done, a true picture may not be obtained As Kosterlitz[23] and others have shown, in malnutrition, the liver cell loses a large proportion of its cytoplasm, although the size of the nucleus remains unchanged A *diminution in enzyme activity per cell* (which, presumably, is what matters) might, therefore, be masked by an increase in number of cells per unit weight At first sight, the solution seems to be to use desoxynucleic acid as a standard of reference In our material, however, this is open to two objections In the first place, it is well known that there is a high degree of polyploidy in the liver Although this is normally less marked in infants than in adults,[24] in our cases, [illegible]

[illegible]

relatively true for the rat, but it is very far from true in man Even in apparently normal livers, as many as one third of the cells are nonepithelial cells, and this proportion may be increased in the absence of any gross pathological lesion It is common in our cases to find infiltrations of lymphocytes in livers that are otherwise histologically normal

For these reasons although we have measured the DNA content of the liver in a number of cases, we cannot regard it as a satisfactory standard of reference The best basis is probably protein nitrogen, provided there is no significant dilution with inert protein, such as collagen Nitrogen figures are not yet available for the cases reported in this paper, and therefore the figures are expressed on the basis of fat free dry weight without correction for glycogen

Results

Of the vast number of possible enzymes, only a few have so far been investigated These have been chosen on two criteria their physiological importance, and their concentration or activity We [illegible] expect to detect changes [illegible] this is approaching the [illegible] reason that we have [illegible] ticularly sensi[illegible]

the baby's admission to the hospital, biopsy of the liver was done by the tran-pleural route The baby was then placed on a high protein diet, consisting mainly of milk, and biopsy was repeated after some four weeks During this time, most cases showed a marked improvement in general condition a gain in weight, although a few were refractory and improved much slowly

During the past three years we have investigated cases of several differe types In some babies the clinical picture is dominated by severe wei loss, with no specific signs, and with a liver which, histologically, shows lit deviation from normal In others there is a fatty liver This is usua accompanied by edema, with low concentrations in the plasma of total prote albumin, and choline esterase These cases may well be regarded as examp of kwashiorkor, although the characteristic lesions of skin and mucosae seem be less common in Jamaica than in some parts of Africa Yet other cases c form to the clinical and pathological picture of serous hepatosis as descri by Hill[6] In these, there is well marked hepatocellular degeneration in liver In general, however, we have avoided cases with evidence of advan liver damage, as the interpretation of the findings is likely to be extrem difficult, and our main interest is in the detection of early changes

In the present paper we have confined ourselves to cases with fatty liv Even in such a restricted group, however, no two patients are exactly ali Until much more material has been accumulated, it is not possible to tr them as a group, to average the results, and to evaluate them statistically in the laboratory We have thought it best, therefore, simply to describ few representative cases which illustrate the results obtained so far in s of the danger of bias inherent in such a method A further difficulty is t of obtaining control material from completely normal children The l that we have been able to do is to use each case as its own control, compar the results before and after treatment

Details of the methods of handling the tissue and of the techniques u for the enzyme assays are g ... metr c meas ments have been done by and Holter[20] This, in cor it possible to do duplicate assays of six or seven different enzyme sys gether with estimations of fat, protein, and nucleic acids, on some 3 mg (fr weight) of liver This amount of tissue is large compared with that used some workers[21] For the present problem, however, there is no advantag further scaling down the amount of liver used On the other hand althou more than 3 mg is often available, it is unwise to count on more than this infants it is not possible to insert the biopsy needle as deeply as in adults that the pieces of tissue obtained are small We also regard it as essential t at least half and if possible more should be put aside for histological exa nation

Before going on to the results it is necessary to consider two techn points of some importance The first is the question of sampling error Th mg of tissue represents only about 1/150,000 of the weight of the whole li

so that clearly our sample may not be representative We have not felt it justifiable to try and get more than one specimen at any given time The best, therefore, that can be done to solve this difficulty is to take two separate fragments from the same specimen (when enough tissue is available) and make two homogenates Admittedly such fragments come from points in the liver only a few millimeters apart, but at least they represent different lobules The histological findings suggest that the pathological processes with which we are dealing are essentially diffuse Although there is some variation, it is from one lobule to another rather than from one large area of the liver to another In general, such comparisons have shown good agreement between duplicate homogenates[21]

The second difficulty is the basis of representation Enzyme activity is usually expressed per milligram dry weight of tissue In our material, dry weight must clearly be corrected for fat, and probably for glycogen also but even when this is done, a true picture may not be obtained. As Kosterlitz[22] and others have shown in malnutrition, the liver cell loses a large proportion of its cytoplasm, although the size of the nucleus remains unchanged A diminution in enzyme activity per cell (which presumably is what matters) might, therefore be masked by an increase in number of cells per unit weight At first sight, the solution seems to be to use desoxynucleic acid as a standard

apparently as a response to injury This fact destroys the basic postulate, that the DNA content per cell is constant Secondly it is generally assumed implicitly by biochemists that the liver is a homogeneous tissue This may be relatively true for the rat but it is very far from true in man Even in ap-

in a number of cases we cannot regard it as a satisfactory standard of reference The best basis is probably protein nitrogen, provided there is no significant dilution with inert protein such as collagen Nitrogen figures are not yet available for the cases reported in this paper and therefore the figures are expressed on the basis of fat free dry weight without correction for glycogen

Results

Of the vast number of possible enzymes only a few have so far been investigated These have been chosen on two criteria their physiological importance and their concentration or activity We cannot expect to detect changes in activity in enzymes with a QO_2 of less than 1 because this is approaching the lower limit of sensitivity of our methods It is for this reason that we have not studied xanthine oxidase an enzyme which seems to be particularly sensi

TABLE 1
COMPARISON OF THE ACTIVITY OF SOME LIVER ENZYMES IN MAN AND RAT

	Man*	Rat
[illegible]	12	140
[illegible]	4	50
[illegible]	23	70
[illegible]		
[illegible] QO_2	55	160
malic, QO_2	207	860
glutamic, QO_2	9	-
Transaminase QCO_2	278	50
Choline esterase, QCO_2	8	9†

* The figures in this column are the average of results obtained in recovered cases
† Female rats

tive to adverse dietary conditions,[11, 12] because even in the rat its activity i
low and in man, so far, we have not been able, in preliminary trials, to meas
it with any accuracy

Since there is almost no information in the literature, the first need is to g
some idea of the pattern of enzyme activity in the normal human liver
has been said, no strictly normal control series is available and therefore
we use as a temporary standard of comparison, or yardstick, the average of
all the figures obtained in recovered human cases The results for eight en
zymes, compared with those found by the same methods in the rat, are shown
in TABLE 1 The difference in activity of the oxidative enzymes on the one
hand, and of transaminase on the other, is too great to be explained by chance
variations of sampling or by errors of technique There seems to be a rea
difference in enzyme pattern between the two species, a difference which
raises problems of some interest It is clearly unsound to consider each en
zyme in isolation The presence of one in high concentration must affec
the activity of others The low level of succinoxidase must, it would seem b
a limiting factor in the activity of the Krebs cycle Transaminases can inter
fere with this cycle by removing or adding substrates at three stages pyruvate
oxalacetate, and α keto glutarate These substances are, as Braunstein put
it[25] at the metabolic crossroads, and may be important links through which
the activity of one enzyme system can control or affect the rate of another
At present, we do not know very much about these interactions, but the great
difference that has been found between rat and man is at least a stimulus t
further investigation It suggests that the normal metabolic paths may, per
haps not be the same in the two species, and supports the point of view alread
put forward, that we must not expect the effects of a given type of malnutritio
to be identical

These considerations form the background to our problem We must no
turn to the effect on activity or concentration of liver enzymes of malnutritio
in general and of that type, in particular, which causes fatty infiltration T
do this, we can draw on the experience of some 30 cases investigated in the pas
but shall present, in detail, only the results obtained in three babies wi
severely fatty livers that have been studied recently (TABLES 2, 3, and 4

TABLE 2

CASE 1 F AGE 6/12 WEIGHT 5.6 KG

	1st biopsy 9.i.53	2nd biopsy 3.ii.53	Average*
Liver fat			
per cent of fresh weight	30.6	6.2	3
fat nonfat solids × 100	255	30	15
Plasma protein gm per 100 ml	3.7	5.7	6.5
Plasma choline esterase Michel units	0.35	0.56	0.6
Succinoxidase QO_2	9.7	4.2	4
Cytochrome-c reductase QO_2	18.5	25.9	23
Dehydrogenases			
lactic QO_2	59	58	55
malic QO_2	320	193	207
glutamic QO_2	10.0	9.4	9
Transaminase QCO_2	264	265	278

* The figures in this column are the average of results obtained in recovered cases

TABLE 3

CASE 2 F AGE 11/12 WEIGHT 6.6 KG

	1st Biopsy 14.i.53	2nd Biopsy 5.ii.53	Average
Liver fat			
per cent of fresh weight	44.7	14.2	3
fat nonfat solids × 100	322	86	15
Plasma protein gm per 100 ml	4.1	6.0	6.5
Plasma choline esterase Michel units	0.50	0.70	0.6
Succinoxidase QO_2	5.1	5.5	4
Cytochrome-c reductase, QO_2	17.1	30.2	23
Dehydrogenases			
lactic, QO_2	24.3	75.0	55
malic QO_2	176	119	207
glutamic QO_2	6.1	18.1	9
Transaminase QCO_2	210	307	278

TABLE 4

CASE 3 M AGE 11/12 WEIGHT 3.5 KG

	1st biopsy 9.x.52	2nd biopsy 20.i.53	Average
Liver fat			
per cent of fresh weight	39.5	3	3
fat nonfat solids × 100	335	19	15
Plasma protein gm per 100 ml	4.5	6.2	6.5
Plasma choline esterase Michel units	0.15	0.61	0.6
Succinoxidase QO_2	0.6	5.2	4
Cytochrome-c reductase QO_2	—	25.2	23
Dehydrogenases			
lactic QO_2	11.3	21.2	55
malic QO_2	185	133	207
glutamic QO_2	10.5	11.7	9
Transaminase QCO_2	425	326	278

From these tables, it can be seen at a glance that, in so far as we are looking for a consistent change in enzyme activity in the fatty liver, the results are negative. Nevertheless, it is worth while to consider each case very briefly in turn, because although all of them had a fatty liver, they showed marked clinical differences in other respects. We shall then try to see what indications the data provide for further lines of attack.

The first case (TABLE 2) was a girl aged 6 months, moderately underweight for her age. She had edema of the feet, hands, and back, with hypoproteinemia. The liver was enlarged and fatty, but there were no other signs of malnutrition, *i.e.* skin hair and mucosae were absolutely normal. Superficially, indeed this child appeared well nourished, because she had an ample cover of subcutaneous fat particularly on the face and limbs. She was, in fact, a perfect example of what has been called the "sugar baby." On treatment with skimmed milk powder, edema rapidly disappeared and the plasma protein concentration rose. The liver diminished in size and lost its fat. Weight gain however was slow. We formed the impression that body fat was being lost while muscle was being laid down. Whether or not this was the explanation, there was a great improvement in general condition in spite of the slowness of weight gain.

The second case (TABLE 3) was more severely underweight, but still retained a fair amount of subcutaneous fat. She had generalized edema, and the liver was enlarged and fatty. The striking difference between this child an first was the presence of very severe lesions of skin and mucosae—glos angular stomatitis and desquamative dermatosis of the legs, arms and p neum. She was a typical case of kwashiorkor, as it is seen in Africa exc that there was no dyspigmentation of the hair, no vomiting, and little diarrhe There was a rapid response to treatment with skimmed milk, edema disap peared and thereafter there was a steady gain in weight. The liver was re duced in size and the skin lesions healed in a few days with no treatment other than milk.

Case 3 differed from the other two in being very severely underweight. Clinically, there was almost complete loss of both subcutaneous fat and of muscle and the child seemed to consist of little more than skin and bone. The skin showed a dry scaly dermatosis, but without the desquamation leaving an underlying raw red surface which was so prominent in Case 2. The hair in parts was quite white like that of an old man. In spite of the low plasma protein concentration there was no edema. The liver was only slightly enlarged but it was extremely fatty, a finding which, in our experience, is unusual in total starvation or marasmus. The response to treatment was very slow and it was only after three months in hospital that a steady weight gain began. Even before this however there was a gradual but undoubted improvement in that indefinable entity the general condition. Nitrogen balance measurements during this period showed a positive nitrogen retention, suggesting that body tissue was being built up gradually, presumably with a concurrent loss of water.

All these three cases had a great increase in liver f

usual method of

expressing fat as a percentag If
fat, F, is calculated as a per here
S = nonfat solids and W = It
seems more illuminating to give the ratio F S, and this we have done in the second line of the tables, taking S as 100 On this basis, the fat content in Case 3 was some 20 times normal, whereas expressed as a percentage of the wet weight it was only 13 times normal

This degree of fatty infiltration represents a gross perversion of metabolism which is not at all reflected in the figures for enzyme activity There was a reduction in the plasma concentration of nonspecific or pseudo choline esterase It has been shown in earlier work[26, 27] that, in such cases, the liver esterase is also reduced, roughly *pari passu* with that in the plasma Low levels of liver and plasma esterase are very constantly found in malnourished infants, but this does not seem to be related in any way to the presence of absence of a fatty liver Rather it seems to be associated with severe weight loss and general malnutrition It is also found, as is well known, when there is advanced structural damage to the liver, as in cirrhosis

The dehydrogenases show rather variable results In the first biopsy, the activity of the lactic enzyme was low in two of the three cases shown This, however, is not a constant finding In three other cases with fatty liver, the initial levels were not low Malic dehydrogenase on the other hand, was present in higher concentration in the first than in the second specimen This has been a rather consistent observation, not only in these, but in other cases also The same is true for transaminase In 11 out of 14 cases, transaminase activity was higher in the malnourished than in the treated liver It would seem that both these enzymes are not only very active, but are well preserved, even selectively preserved It would clearly be unwise in work of this kind, to confine our interest to enzymes which are reduced by dietary deficiency The fact that some particular enzymes are jealously retained may equally be of significance The figures for glutamic dehydrogenase activity show much scatter, and we have not yet been able to draw any conclusions from them

So far, not very many measurements have been made of cytochrome reductase activity It is too soon to attach any significance to the slightly low figures found in the initial biopsies of Cases 1 and 2 The results are included here in order to demonstrate one point only that, in Case 2, there was a fair concentration of this flavoprotein enzyme in the liver in spite of the presence of severe signs which clinically are attributed to ariboflavinosis

In the case of succinoxidase, the results again are variable A very low activity, however, like that found in Case 3 has been observed in some other babies who were severely malnourished with or without fatty liver We cannot feel the same confidence about these results as in the case of the other enzymes, because on two occasions there has not been satisfactory agreement between duplicate homogenates To admit the possibility of technical error or artifact need not however be entirely a confession of failure Artifacts can be significant The instability of the Krebs cycle enzymes probably arises from the fact that they are parts of an organized structure parts which cannot

work if their interrelationship is disturbed Succinoxidase, although less labile than the others, is a member of this group both functionally and structurally since it is found only in the mitochondria In the assay of such enzymes since some degree of organization must be preserved, we are one step nearer to conditions *in vivo* than when measuring the activity of a soluble enzyme such as transaminase It is conceivable that the effect of malnutrition (that is, of an adverse chemical environment *in vivo*) may be not to reduce the amounts of enzyme molecules but, in some way, to destroy their organization or to make it more easily destroyed under the conditions of the *in vitro* measurements

particulates is not the same in the two species Histologically, both mitochondria and basophil substance are much more sparse in the human liver than in that of the rat This is confirmed by preliminary measurements that we have made of the ribonucleic acid content of the liver [29] In our cases, this was very low The ratio of RNA to DNA was about 1 1, compared with about 4 1 in the rat Because of the doubt raised by Rossiter and his colleagues[28] about the specificity of the color methods, and because of the possibilities of artifact arising from working in a tropical climate without a cold room, we have postponed pursuing this investigation further until a critical examination of the methods has been completed For this reason, we do not present here data on the nucleic acid content of the fatty liver But the point has been raised in order to emphasize again the need for more basic information about the human liver

In conclusion, in these fatty livers the activity of those enzymes which have been measured is, on the whole, well maintained The only enzymes which have been found, in these and other malnourished cases, to be reduced in amount are the nonspecific choline esterase and perhaps succinoxidase These changes seem, however, not to be related to the fatty liver *per se*, but to the general state of malnutrition of the infant There may well be a connection between such a reduction in enzyme activity and the semi irreversible state which some of these infants seem to reach when malnutrition has been sufficiently severe or prolonged The fatty liver is only one element in this picture, and not necessarily the most important Although the term 'fatty liver disease'[3] has its uses (it might be a more appropriate label for Case 1 than kwashiorkor), it must not be used to imply, as one of us did five years ago[1] that the fatty liver is the dominant feature on which a prognosis can be based. This position can no longer be held When these babies die, in all likelihood it is not the fatty liver that kills them, but something else which, for want of a better term, we can only call generalized protein malnutrition The severity of this does not necessarily run hand in hand with the degree of fatty infiltration in the liver

Since there are many variables in this clinical picture, the collection of facts that can be classified compared and analyzed, is a slow process We must apologize for presenting, in the meantime, a piecemeal account of work that

has been done on such a small number of cases Our justification is the hope that others will pursue this path, so that knowledge may be gained more quickly

Appendix

Methods

The liver specimens are obtained with a Vim Silverman needle inserted by the transpleural route The infant is given paraldehyde *per rectum* before hand and 2 per cent novocaine is used to infiltrate the skin at the point of incision The tissue is transferred from the needle into ice-cold saline A

there is enough tissue to make duplicate homogenates, and each piece is weighed on a quartz fibre torsion balance The balance that we use was constructed in the laboratory according to the principle illustrated by Strong[29] The only modification is that we do not fuse the fibre to the beam, which is extremely difficult but seal it with a special resin (Araldite, made by Aero Research Ltd, Duxford Cambridge, England) This has proved very satisfactory The zero of the balance has remained unchanged for 18 months The only dis advantage of this method is that it is not possible to coat the balance with platinum by painting with chloroplatinic acid and flaming in order to prevent the accumulation of electrostatic charges However, at the level of sensitivity at which we work, and in the rather humid climate of the tropics, we have not had any trouble from this source We have not aimed at a sensitivity greater than 2 μg With 2 mg of tissue the error of weighing is still much less than the other errors of the chemical or enzymatic measurements

One piece, after weighing, is put over phosphorus pentoxide to dry, and re weighed the next day, to give the water content This piece should if possible weigh at least 1 mg, fresh weight, or ¼ mg dry weight, although the chemical measurements can be done with less The dried liver is put into a micro Soxhlet apparatus similar to that described by Kirk[10] extracted with hot ether, dried and weighed again This method of measuring fat content by differ ence after ether extraction gives results that are a little low It is certain that some of the phospholipid is left behind In the normal liver, this method gives a fat content of about 3 per cent of the wet weight, compared with the accepted normal value of 5 per cent The difference is consistent, however, and is of little importance for our purpose since the neutral fat does seem to be taken out quantitatively

In the past we have used this dried defatted tissue for measurements of nucleic acids and of protein nitrogen The dry specimen was homogenized and extracted with hot trichloracetic acid, according to the method of Schneider[3] The final volume of extract was about 30 cu mm Total nucleic acids were measured in the extract by ultra violet absorption after diluting 25 times with water In this way, the TCA blank becomes negligible DNA was measured by the diphenylamine reaction As this reaction is not very sensitive it was

necessary to have a final volume of not more than 20 cu mm., and this involved using a microscope colorimeter[30] RNA was measured by difference After extraction with TCA, protein nitrogen was measured in the residue by digestion and nesslerization

The other piece of tissue is used for the enzyme assays, and should weigh not less than 2 mg We homogenize in a volume of 20 cu mm, and aim at a tissue concentration of about 10 per cent If less than this is used the accuracy of measurement suffers for those enzyme systems, like succinoxidase, which have a low QO_2 The tissue is homogenized in ice cold 0 88M sucrose in a tube 2 to 3 mm in diameter The pestle of the homogenizer is made of glass and is rotated by a motor The conditions of homogenization are kept as standardized as possible During homogenization, which lasts only for 30 seconds, the tube is held in a large brass block previously cooled on ice

Once the homogenate has been made, it is necessary to work quickly Of the enzymes investigated so far, we have not found any loss of activity in *intact* tissue that has been left on ice for several hours, but in a homogen some enzymes do become inactivated at an appreciable rate on stand notably succinoxidase The dehydrogenases, transaminase and ch esterase seem to be stable for many hours Immediately after the homoge has been made aliquots are removed and diluted for the assays of dehydro ases and of cytochrome c reductase For the former, the dilution is n in water, with a tissue concentration of about 5 µg per cu mm. For the la the dilution is made in 0 88M sucrose with a tissue concentration of abo µg per cu mm Two sets of measurements can then be done simultaneo one by spectrophotometric the other by gasometric methods All the g metric measurements have been made by the Cartesian diver techniqu Linderstrøm Lang and Holter, as modified by Waterlow and Borrow,[32] an Borrow and Penney[33] This technique is sensitive, accurate, and vers and the apparatus neede time needed to fill the di to fill and measure more to 60 minutes, the limitations of fatigue make it difficult for one person to more than about 16 divers in one day

Assay Methods

Gasometric All assays have been done at least in duplicate In the instance the conditions chosen have been those which have been worked for assays on rat liver Whenever enough time and enough tissue have l available, measurements have also been made with varying concentrat or substrate buffer etc since it cannot be assumed that the optimum cond t are the same for man and the rat

Succinoxidase The method was that of Schneider and Potter[34]

Diver filling (FIGURE 1) *Bottom drop* 0 5 µl of 2N NaOH *On the wall* 0 25 µl of a mixture containing cytochrome c (Wyeth) 2 5 mg per $AlCl_3$ $3 \times 10^{-3}M$ $CaCl_2$ $2 5 \times 10^{-3}M$ *Homogenate* 1 µl of sucrose homo ate, strength about 10 per cent To this was added 0 25 µl of a mixture

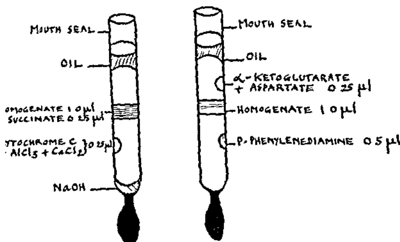


FIGURE 1 (left) Filling for succinoxidase assay FIGURE 2 (right) Filling for transaminase assay

drop containing cytochrome[24] Readings were made for one hour A blank was always run containing all reagents except succinate because at this high tissue concentration the endogenous respiration is appreciable (QO_2 = 1 to 2)

Transaminase Our method is essentially that of Ames and Elvehjem,[25] in which the reaction measured is the formation of glutamic and oxaloacetic acids from aspartic and α keto-glutaric acids The oxaloacetate is then decomposed, and the CO_2 output measured It was found however that activity was often not linear in relation to the amount of tissue being less with large amounts The same effect was found when the time of incubation was varied, the activity being relatively less with incubations of 15 or 20 minutes than of 5 or 10 minutes It is probable that this arises from the position of the equilibrium point in this reaction, which is reached when about ⅓ of the aspartate and *α-keto-glutarate have reacted*[26] The effect has been *reduced but not* eliminated by increasing the concentration of substrates The strength of the homogenate has been kept as constant as possible and measurements have been made in duplicate with only one strength of tissue incubated for 5 and 10 minutes For decomposing the oxaloacetate we have used a solution of p phenylenediamine instead of aniline citrate With this, evolution of CO_2 is complete in about 5 minutes Aniline citrate has the disadvantage of being oily, so that it cannot be placed as a discrete drop on the diver wall

Diver filling (FIGURE 2) *Lower drop* 0 5 µl of M/1 p-phenylenediamine in N/1 HCl *Homogenate* 1 µl of homogenate diluted to 2 per cent with 0 88M sucrose *Upper drop* 0 25 µl of a mixture containing aspartate 0 3M, α keto-glutarate 0 3M phosphate buffer 0 15 M pH 7 4 *Homogenate and*

upper drop mixed by applying a negative pressure after equilibration. Low drop mixed by applying a positive pressure 5 or 10 minutes later CO_2 outp is complete in 5 to 10 minutes

For the blank exactly the same filling is used, but the two mixings are carri out in immediate succession so that the incubation time is zero

Choline esterase Diver filling 1 25 μl of homogenate To this is add during the filling of the diver 0 25 μl of 0 19M $NaHCO_3$ and 0 3 μl of 2 5 p cent acetyl choline Gas-phase 5 2 per cent CO_2 in oxygen Divers gass under water by the technique of Waterlow and Borrow[32] Readings are tak from 15 to 45 minutes after the moment of adding the acetyl choline

Spectrophotometric Methods

Dehydrogenases The lactic, malic, and glutamic dehydrogenases w measured by following the reduction of DPN Density measurements w made at 340 mμ with the Beckmann spectrophotometer The details of t method were kindly supplied by Dr J S Strominger of the National Institut of Health The amounts of tissue used for the assays were of the order 5 μg for the lactic dehydrogenase, 2 μg for the malic dehydrogenase, and 50 for the glutamic dehydrogenase The final volume was, in all cases, 0 110 Results are expressed in terms of QO_2 This calculated from the observ density changes by the use of Ohlmeyer's figure of 6270 for the molecu extinction coefficient of $DPNH_2$[37]

DPN-Cytochrome-c Reductase This enzyme was measured by a mic adaptation of the method of Brody *et al*[38] In this method the amount of duced cytochrome c is determined by measuring the optical density at 550 The amount of tissue used was of the (
0 110 ml Results are expressed as QO
observed density changes by the use of t
lecular extinction coefficients of oxidized and reduced cytochrome c[39]

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THE QUESTION OF THE RELATIVE IMPORTANCE OF PROI AND LABILE METHYL IN THE DEVELOPMENT OF FATT LIVER AND CIRRHOSIS IN MAN

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The hypothesis that fatty liver disease and cirrhosis in man is caus
nutritional deficiency has never been proved,[5, 10] but many clinicians wc
in temperate climates hold the view that the lesion develops in alcoholics
they cease to ingest a good mixed diet Investigators working in the t
believe that a low intake of protein is an important factor in the causati
"kwashiorkor" in children[19, 23, 2] Trowell[20] has stated that animal p
is particularly important in this respect

Animal experiments have shown that at least part of the lipotropic
of protein is the resultant of the opposing influences of cystine and methi
that a deficiency of choline leads to fatty liver, and that hepatic fibrosis r
from chronic interference with the utilization of methionine[6] Other nu
interrelationships are involved in the production of fatty liver in an
Cystine exerts a positive effect on the accumulation of liver fat only if th
is low in niacin,[22] and vitamin B12 and folic acid have been shown to infl
the metabolism of choline and methionine[16] Some evidence suggests
unidentified factors may be present in the protein molecule which affect
fat deposition[21, 3, 1] A factor, as yet unidentified, has recently been pu
from casein and from certain yeasts which protects rats against dietary ne
liver degeneration[17, 18]

The present communication deals with the incidence of hepatome
fatty liver and cirrhosis in three West African tribes and in one group of ra
similar wealthy native traders as related to their diets and other environm
factors

Methods and results A general description of the investigations carrie
in Nigeria between 1947 and 1951, and the methods used to determine nu
intakes of Nigerian natives, have been published elsewhere[12, 13] The sa
of the tribes were formed of all the inhabitants estimated to be over t
years of age in three small villages in the tribal areas The traders lived
town of some size in the center of the Niger Delta Clinical enlargeme
the liver was diagnosed if the organ was palpable two fingers below the c
margin in the midclavicular line, the subject lying on his back and brea
normally Ascites and jaundice were encountered occasionally in associ
with hepatomegaly, gynecomastia and feminism were seen occasional
males, spider naevi were noted in a few natives who had pale skins and
cular wasting was a prominent feature of the more advanced cases H
tomegaly was found at all ages but more frequently after the third de
The lesion was observed more often in females than in males, but the diffe

* I am grateful to the Inspector General of Medical Services Nigeria for permission to publish this
and to Doctor Klaus Schwarz National Institutes of Health U S Public Health Service Bethesda M
help in its preparation

as not statistically significant Infective hepatitis is a common disease hroughout Nigeria Such cases of liver enlargement were excluded from his study

Specimens of liver tissue were obtained by needle biopsy or by laparotomy [illegible]

n the sections

The incidence of hepatomegaly in the four groups, together with the prevalent parasitic infestation rates, the calculated daily intake of alcohol, and the frequency of a past history of transient jaundice are shown in TABLE 1 The consumption of alcohol, a habit freely indulged by men, women, and often children, is highest in the traders, in which group the incidence of enlarged liver s least The total parasitic load is approximately equal in the three tribes, and it is believed that nutritional demands made, and any hepatic damage caused, by parasitic toxins or the parasites themselves, are likely to be of the same degree in each tribe Clinical disease and pathological states attributable to specific parasites are seen less often than would be expected from the numbers of natives who carry these organisms The incidence of hepatomegaly is

TABLE 1

THE INCIDENCE OF HEPATOMEGALY THE PREVALENT PARASITIC INFESTATION RATES THE DAILY ALCOHOL CONSUMPTION, AND THE FREQUENCY OF A PAST HISTORY OF TRANSIENT JAUNDICE IN THREE WEST AFRICAN TRIBES AND IN ONE GROUP OF WEALTHY TRADERS

	Traders (90)*	*Ijaws* (112)*	*Isokos* (123)*	*Dakarkerris* (104)*
	%	%	%	%
Incidence of hepatomegaly	3	8	19	0
Parasites ‡				
Schistosomes	1	0	2	43
E histolytica	4	8	3	3
Ankylostomes	73	96	95	63
Ascaris	17	20	31	5
G lamblia	3	4	2	6
Dracontiasis	0	0	0	7
Filaria				
loaloa	4	4	3	0
bancrofti	0	0	0	3
Splenic index (children 2-10 years)	17	17	22	17
Previous history of transient jaundice	3	8	6	6
	ml	ml.	ml	ml
Alcohol consumption—average daily intake of				
Home brewed beer (2-5%)	0	0	0	228
Palm wine (1-7%)	120	105	250	0
Local spirits (60-70%)	20	107	13	0
Imported beer (4-6%)	100	0	0	0
Imported spirits (50-70%)	64	0	0	0

* Numbers examined clinically
† Figures in parentheses denote alcoholic content
‡ Number of individuals found to be carriers of parasite or its ova not all suffering from specific disease as a result of the infestation

TABLE 2

CALCULATED DAILY INTAKE OF NUTRIENTS OF THREE NIGERIAN TRIBES AND OF ONE G[illegible]
OF WEALTHY TRADERS

Group	Protein An	Protein Veg	Fat	ChO	Vitamin A*	Thiamine	Riboflav	Niacin	Vit. C	Fe	Ca
	g	g	g	g	I U	mg	mg	mg	mg	mg	mg
Traders	50	34	86	473	9200	1 02	1 37	18 1	71	21	1302
Ijaws	68	12	39	379	5700	0 30	0 68	12 1	40	24	2800
Isokos	20	26	43	424	7400	0 69	0 65	8 9	30	21	664
Dakarkerri	2	85	24	595	700	3 70	0 98	25 0	34	57	581

	Contribution to calories (%)			Composition of diets by weight		
	Protein	Fat	Carbohydrate	Protein	Fat	Carbo[illegible]
Traders	11 2	25 8	63 0	13 1	13 4	73
Ijaws	14 6	16 4	69 0	16 1	7 8	7[illegible]
Isokos	8 1	17 1	74 8	9 0	8 4	8[illegible]
Dakarkerri	11 8	7 3	80 9	12 3	3 4	8[illegible]

* 1 i u of Vitamin A taken as equivalent to 3 i u of Vitamin A in the form of carotene
No allowance made for destruction of nutrients by cooking

least in the traders (3 per cent) and greatest in the Isoko (19 per cent) corresponding figures for the Ijaws and Dakarkerris being 8 per cent and cent respectively, the difference between the rate in the Isoko and in other two tribal groups being significant (P = 0 01) A correlation bet hepatomegaly on the one hand and a previous history of jaundice or any sitic infestation on the other hand was not found

Full details of the foodstuffs eaten are given in the appendix The culated daily intake of nutrients is given in TABLE 2 It will be seen tha amounts of individual nutrients vary widely from group to group, resu from the use of different staple foods in the different regions of Nigeria traders' diet is considered to be reasonably adequate, but the three trib ets each show grave deficiencies of one or more nutrients A low inta protein, however is the only feature by which the Isoko diet is distingu from the diets of the other two tribes The Isokos ingest 46 gm of pr daily, of which 20 gm is derived from animal sources, the Ijaws' diet [illegible] Dakarkerris e[illegible]

[illegible] is calculated numerous analyses of the amino acid content of foodstuffs, choosing an age according to species, soil, and climate For these analytical data, I a debted to Doctor M Autret of the Food and Agricultural Organization ficient data applicable to African foodstuffs has not been found from whi assess the cystine content of the diets The results, together with the am of protein nitrogen ingested,[4] are given in TABLE 3 Comparison is mad tween the essential amino acid composition of the Nigerian diets and th quirements of man advocated by Rose[16] TABLE 3 also includes an estima

TABLE 3

Nutrient	Rose (1949)		Traders	Ijaws	Isokos	Dakarkerris
	Minimum*	Allowance				
Calories	3600	3600	3000	2200	2300	2900
Protein (g)						
Animal			50	68	20	2
Vegetable			34	12	26	85
Nitrogen† (g)	10	10	13	12	7	13
Essential amino acids (g)	6 4	12 8	30 3	28 8	15 8	27 2
Iso-leucine	0 7	1 4	4 1	3 8	2 0	3 6
Leucine	1 1	2 2	6 5	5 8	3 2	8 3
Lysine	0 8	1 6	5 3	5 8	2 7	2 1
Methionine	1 1	2 2	2 0	2 2	1 2	1 3
Phenylalanine	1 1	2 2	3 5	3 0	1 9	3 4
Threonine	0 5	1 0	3 4	3 3	1 8	3 1
Tryptophan	0 3	0 5	1 0	0 8	0 6	0 9
Valine	0 8	1 6	4 5	4 1	2 4	4 5
Choline (g)			0 92	0 65	0 60	0 65
Incidence of hepatomegaly (%)			3	8	19	9

* Minimal requirements for maintenance of nitrogen in healthy young American adult males

† Does not include any nonprotein nitrogen present in the foodstuffs used

the choline content of the African diets, which has been made from such published data as are available[6, 7, 8, 11]. For some of the foods, the choline and amino acid contents had to be derived by analogy

If Rose's daily recommendations are considered, the only deficiencies of essential amino acids detectable are those of methionine in the diets of the traders, Isokos and Dakarkerris and of phenylalanine in the Isoko diet Of the eight essential amino acids, each group ingests more than Rose's minimal requirements for the maintenance of nitrogen balance in healthy adult Ameri

reason does not exist to suppose that the Dakarkerri diet contains considerably more cystine than that of the Isokos

Discussion The data presented above show that the only feature whereby

Enlargement of the liver undoubtedly results from invasion of the organ by parasites as in amebic hepatitis and schistosomiasis and part of the total incidence of hepatomegaly in all groups is due to such causes but these lesions

are manifested by focal necrosis and abscess formation, patchy fibrosis and associated regeneration of liver tissue, not by fatty infiltration and uniform cir rhosis A large percentage of the sections of liver from individuals sufferi from hepatomegaly in this series showed the latter picture It has be pointed out above that the incidence of parasitic infestations does not paral the incidence of hepatomegaly, and it is believed that nutritional factors accou for the varying incidence of this clinical feature in the tribes under conside tion

In view of the above discussion, and as a low intake of protein is the o feature by which the Isoko can be distinguished from the other groups c sidered, a further argument is advanced to explain the high incidence hepatomegaly in this tribe namely, that some unidentified factor, which occ in, or is associated with, protein is involved in the protection of the liver fr fatty infiltration and cirrhosis, and possibly also from the effects of paras toxins and alcohol The existence of such a factor has been suggested in literature cited in the introduction to this paper Such a factor would app to be present and active both in vegetable and animal protein and theref is unlikely to be related to vitamin B12

Summary (1) The incidence of hepatomegaly (as defined) in three gerian tribes and in one group of wealthy native traders is recorded toget with a description of the diets eaten, the histological appearance of the li lesions, the prevalence of parasitic infestations, and the alcohol consumpt of each group

(2) The importance of dietary protein in the etiology of the disease is cussed The evidence obtained does not support the view that lack of of the essential amino acids or lack of labile methyl in the diet is of importa in the development of fatty liver and cirrhosis in the groups investigated.

(3) It is suggested that some unidentified factor, which occurs in asso tion with both animal and vegetable protein, plays a part in preventing development of fatty liver and cirrhosis in man

Appendix

Average Daily Food Consumption of Nigerian Peasants and Trad (Adults)

Foodstuff	Scientific name	Traders	Ijaws	Isokos	Da
		g	g	g	
Wheat flour (70%)	Triticum vulgare *Host* Cultigen	70	—	—	—
Rice milled	Oryza sativa Linn	76	—	—	(
Rice home pounded	Oryza sativa Linn	—	—	—	—
Maize mature yellow	Zea mays Linn	30	29	15	1
Maize immature		10	—	7	76
Guinea corn	Sorghum vulgare Linn	—	—	—	22
Bulrush millet	Penisetum typhoideum Rich	—	—	—	

APPENDIX (*continued*)

AVERAGE DAILY FOOD CONSUMPTION (*continued*)

Foodstuff	Scientific name	Traders	Ijaws	Isokos	Daks
		g	g	g	g
Cassava	Manihot utilissima Pohl				
flour		109	186	143	5
starch		102	92	172	—
'fufu'		—	—	63	—
fresh tuber		—	77	—	—
Yam	Dioscorea sativa Linn	300	63	633	—
Cocoyam	Colocasia antiquorum Schott	—	12	—	4
Kaffir potato	Plectanthus sp	—	—	—	15
Plantain green	Musa paradisiaca Linn.	17	13	14	—
Banana	Musa sapientium Linn	22	—	—	—
Sweet potato	Ipomoea batatas Linn	—	19	—	—
Cow pea	Vigna sinensis	15	—	13	15
Jak fruit	Artocarpus integer Merr	—	—	3	—
Groundnuts	Arachis hypogaea Linn	4	—	2	—
Locust bean cake	Parkia filicoidea Welw	—	—	—	1
Pumpkin seed	Cucurbita spp	3	—	1	—
Coconut kernel	Cocos nucifera Linn	2	—	—	—
Oil bean kernel	Pentaclethra macrophylla Bth	—	—	1	—
Dika nut kernel	Irvingia gabonensis Baill	1	—	—	—
Kola nut kernel	Kola accuminata Schott & Endl	3	1	1	1
Shea nut husk	Butyrospermum parkii	—	—	—	45
Shea nut oil		—	—	—	1
Onions	Allium cepa Linn	4	—	—	—
Okra fresh	Hibiscus esculentus Linn	6	—	—	2
Okra dried		—	—	—	1
Red sorrel fresh	Hibiscus sabdariffa	—	—	—	1
Red sorrel dried		—	—	—	5
Green leaves	Corchorus spp amaranths and others	12	—	—	—
Peppers	Capsicum frutescens Bl				
red					
dry		3	4	3	2
fresh		2	1	3	—
green fresh		5	1	2	—
Baobab leaf fresh	Adansonia digitata B Juss	—	—	—	16
Baobab fruit		—	—	—	1
Tomato fresh	Lycopersicum esculentus Mill	4	—	2	—
Avocado pear	Persia americana Mill	26	—	—	—
Mango	Mangifera indica Linn	20	—	2	45
Taura	Detarium senegalense Gmel	—	—	—	1
Guava	Psidium guajava Linn	4	—	—	—
Pawpaw	Carica papaya Linn	12	—	—	—
Orange	Citrus sinensis sp	10	—	—	—
Custard apple	Annona reticulata Linn	7	—	—	—
Fish mixed estuary and river					
dried		28	78	18	1
fresh		62	165	18	—
Beef		96	—	—	—
Mutton		12	—	—	—

Appendix (*continued*)
Average Daily Food Consumption (*continued*)

Foodstuff	Scientific name	Traders	Ijaw
		g	*g*
Pork, fat		16	—
Goat		—	—
Monkey, dried	Various species	—	—
Rat meat	Cricetomys gambianus	—	—
Rat meat	Thryonomys swinderianus	—	—
Pangolin	Manis longicaudata	—	—
Porcupine	Atherurus africanus	—	—
Frogs' legs	Rana mascarenensis	—	—
African snail	Archachatina sp	3	—
Palm weevils	Rhyncophorus phoenicis	1	—
Snake meat	various species	—	—
Shrimps, dry		2	1
Prawns, fresh		—	4
Oysters, local		—	7
Liver		4	—
Eggs		10	—
Caterpillars		—	—
Sugar cane stem	Saccharum officinarum Linn	26	4
Palm oil	from Elaeis guineensis Jacq	36	27
Margarine, imported		5	—
Jam, imported		8	—
Sardines, tinned		3	—
Milk			
sweetened condensed		5	—
evaporated		28	—
local, sour		—	—
Palm wine (ml)	from Elaeis guineensis and Raphia vinifera	120	103
Native beer (ml)	from sorghum vulgare and penisetum	—	—
"Illicit gin" (ml)		20	107
Gin, imported (ml)		26	—
Whiskey (ml)		23	—
Brandy (ml)		15	—
Beer, imported (ml)		100	—
Tea		4	—
Coffee		3	—
Nut (Urhobo "ewoie")		—	1
Native salt		4	4
Imported salt		10	7

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RELATIVE EFFECTS OF PROTEIN AND LIPOTROPIC SUBSTANC IN THE TREATMENT OF NUTRITIONAL CIRRHOSIS IN RATS*

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It is established that rats fed diets low in protein and deficient in l substances develop fatty changes and cirrhosis of the liver It has also shown that the addition of choline and methionine to these diets prevent development of the lesions Fewer studies have dealt with the treatme nutritional cirrhosis once it is fully developed

The first reports on treatment of dietary cirrhosis in the rat were the Lowry, Daft, Sebrell, Ashburn, and Lillie[1, 2] After producing cirrho weanling rats with a diet containing 4 per cent casein, liver biopsies wer formed, after which the animals were placed in two therapeutic group receiving the basal diet plus 40 mgm of choline daily, the other recei high casein diet containing either 30 or 50 per cent casein In a short study, animals were sacrificed at intervals up to 42 days and, in a longer study, at intervals up to one year Comparison was made with the biopsies In both groups, the addition of choline to the basal diet an change to a higher casein diet brought about disappearance of fat reg tion of liver cells, but no apparent decrease in the amount of connective ti

Sellers, Lucas, and Best[3] tested various dietary regimens on young rats with cirrhosis of the liver induced by carbon tetrachloride pois After a moderate degree of cirrhosis was produced, a representative samp sacrificed for control studies, the remainder being placed on the test di 12 to 13 weeks In these experiments, the addition of choline, of meth or an increased proportion of casein brought about disappearance of f generation of liver cells, and loss of connective tissue as well These seemed to be equally effective It was concluded, therefore, that the r tive effects were attributable largely to lipotropic substances

In a more recent report by Gyorgy and Goldblatt,[4] employing a large of rats doubt was thrown on the effectiveness of lipotropic substances particularly in advanced stages of the disease Because of the high mo involved, biopsy technique was abandoned, and their observations were largely upon sampling after 75 to 150 days of treatment In those a with moderate degrees of cirrhosis lipotropic substances seemed to e curative effect, whereas in more advanced cirrhosis, the addition of thes stances to the basal diet was much less effective Best results were ob by a combination of casein and liver extract

The problem was re examined because of the divergent results In t periments to be described nutritional cirrhosis was produced in the ra diet patterned after that of Daft, Sebrell, and Lillie[5] An attempt was m

* Permission has been granted by the Journal of Experimental Medicine to reprint TABLES 1 2 and report.

[illegible] protein and of lipotropic factors separately and [illegible]ease process These studies suggest that high [illegible]urative effects greater than those attributable to choline and methionine

[illegible]

[illegible] a 30 per cent [illegible] [illegible]

and biopsy technique, despite the attendant mortality

[illegible]

and weekly supplements of percomorph oil and alpha tocopherol The animals were permitted to feed *ad libitum*

The plan of the experiment is shown in TABLE 2 Choline was mixed with the diet in a proportion of 0 5 per cent The average daily consumption per rat was 40 mgm of choline Methionine was added at a level of 0 8 per cent The average daily consumption per rat was 64 mgm This was estimated to be equivalent to the methionine content of the 30 per cent casein diet

Results The caloric intake was essentially the same for those on the 4 per cent and 30 per cent casein diets, but their rates of growth varied markedly,

TABLE 1
COMPOSITION OF EXPERIMENTAL DIETS

Low-protein	*per cent*	High protein	*per cent*
Vitamin free casein	4 0	Vitamin free casein	30 0
L-cystine	0 5	L-cystine	0 5
Wesson oil	5 0	Wesson oil	5 0
Salt mixture	3 0	Salt mixture	3 0
Corn starch	87 5	Corn starch	61 5

TABLE 2
PLAN OF EXPERIMENT

Group no	Foreperiod (16-19 wks.)	Treatment period (15 19 wks)	
1	4 per cent casein diet	Biopsy	4 per cent casein diet + choline + methionine
2	4 per cent casein diet	Biopsy	30 per cent casein diet + choline
3	4 per cent casein diet	Biopsy	30 per cent casein diet
4	4 per cent casein diet (control)	Biopsy	4 per cent casein diet
5	30 per cent casein diet (control) + choline	Biopsy	30 per cent casein diet + choline

those on the 30 per cent casein diets showing a sharp acceleration of grow
Unexpl-
30 per
unsatur
dermatitis was but partially arrested Of 143 rats employed, 69 died du
the foreperiod In this early stage, death was due chiefly to hemorrh
cortical degeneration of the kidneys, presumably from choline deficiency \
others died within one week of the diagnostic biopsy There remained 0
suitable for testing various therapeutic diets

During the period of treatment, there was a sharp reduction in death ra
all groups, once the animals survived a critical period of about two weeks. T
death rate was higher in those fed the 4 per cent casein diet supplemented w
choline and methionine than in those fed 30 per cent casein diets

Comparison was made of histologic sections obtained at the time of bio
and at autopsy Only those specimens were compared in which animals
ceived treatment for at least 10 weeks Sixty animals satisfied this criterion

The results are shown in TABLE 3 The tabulation is restricted to fatty
filtration and changes in connective tissue, since other changes are difficult
compare in a quantitative fashion Certain trends seem evident. For

per cent casein, with or without added choline Fibrosis appeared to incre
in rats on the 4 per cent casein diet, despite the addition of choline and met
onine In contrast, those rats whose diets were changed from 4 to 30 per ce
casein showed either moderate regression (group 2) or arrest (group 3) of t
process In this small group, it is doubtful whether the differences betwe

TABLE 3
HISTOLOGIC APPEARANCE OF LIVER AS DETERMINED FOR SPECIMENS AT BIOPSY AND AUTOPSY*

Group No			Fatty infiltration		Connective tissu	
			Biopsy	Autopsy	Biopsy	Autop
1	4 per cent casein + choline + methionine (18 rats)	Absent	0	4	2	2
		Minimal	3	10	8	3
		Abundant	15	4	8	13
2	30 per cent casein + choline (21 rats)	Absent	2	10	1	7
		Minimal	8	11	13	7
		Abundant	11	0	7	7
3	30 per cent casein alone (12 rats)	Absent	0	7	1	1
		Minimal	3	4	6	6
		Abundant	9	1	5	5
4	4 per cent casein control (4 rats)	Absent	0	0	1	0
		Minimal	0	0	2	0
		Abundant	4	4	1	4
5	30 per cent casein control (5 rats)	Absent	5	5	5	5
		Minimal	0	0	0	0
		Abundant	0	0	0	0

* Only those specimens are compared in which animals received dietary treatment for 10 or more weeks

[illegible]

amino acid composition of casein Such amino acid mixtures appeared to be as effective in promoting repair of the liver as any of the diets employed above

Evaluation of results is always difficult, and comparison with other studies is hazardous because of different experimental conditions There are several possible explanations Possibly methionine contained in the 30 per cent casein diet is more effectively utilized than when it is added as a supplement to the low casein diet Possibly other amino acids play a rôle in the reparative process

In conclusion, within the conditions of these experiments, the studies suggest [illegible]

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Discussion of the Paper

Doctor P György, Philadelphia It is too much to ask to expect identical results with beneficial prophylactic measures in the therapy of the same condition, such as cirrhosis In prophylactic experiments, the effective factors should and will prevent the development of the major specific metabolic and anatomical changes Therapeutic efforts, on the other hand, have to deal with the arrest or even regression of already existing metabolic and anatomical disturbances For instance, in the case of cirrhosis, the progress of fibrosis has to be checked or even reversed, in addition to the repair of all other concurrent pathologic manifestations Furthermore, in cirrhosis, not only the liver but often other organs as well, such as the kidney and endocrine organs, among the latter especially the gonads, are found to be involved in the over all disease In decompensated liver disease, high protein diet may even be injurious just as exercise may be harmful in decompensated heart disease Protein, or lipotropic substances may be beneficial in the further progression of the disease and perhaps in the recovery stage

PROTEIN METABOLISM IN PATIENTS WITH CIRRHOSIS OF THE LIVER*

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The pathogenesis of hepatic cirrhosis is, in some measure, related to prot malnutrition Contrariwise, established liver disease may affect nutr including the metabc
observations relating
rhosis of the liver
cerning this aspect of nutrition in liver disease, and a presentation of data n previously reported The presentation of data derived primarily from laboratory is done with a full realization of the valuable contributions of ot many of which have been cited in previous publications

The data presented were obtained from the study of patients the Thorndike Metabolic Ward where specialized dietary and nursing are available All of the patients studied had advanced cirrhosis of the associated with chronic alcoholism and an inadequate dietary intake T presented obvious clinical and laboratory evidence of parenchymatous disease in varying stages of activity and chronicity In many instances t clinical diagnosis was confirmed by needle biopsy of the liver, or at necrops

All of the patients were allowed *ad libitum* ambulation on the ward, no being confined strictly to bed. None presented clinical or laboratory ev dence of cardiac or renal disease, nor was any study complicated by vomiting diarrhea, gastrointestinal bleeding or febrile illness Although anorexia m be a prominent symptom of liver disease, this factor does not influence the me tabolic data presented since the patients were maintained on constant intake of food as indicated The methods of metabolic study, the various analytic procedures, and the clinical laboratory tests involved have been described in previous publications

Consideration of the effect of well established cirrhosis of the liver upo the metabolism of protein is presented in the following sequence (1) the me tabolism of dietary protein, (2) the utilization and excretion of amino acid (3) the untoward consequences of the administration of certain nitrogenou substances, and (4) finally an evaluation of these data as they apply to th dietary management of patients with this disease

1 *Protein*

That digestion and absorption of ingested protein is normal in patients wi cirrhosis of the liver is indicated in FIGURE 1, which demonstrates that st nitrogen excretion is not increased above normal over a range of prote intakes of from 0 to 100 gm daily For example the patients ingesting 0 gm

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† Welch Fellow in Internal Medicine of the National Research Council

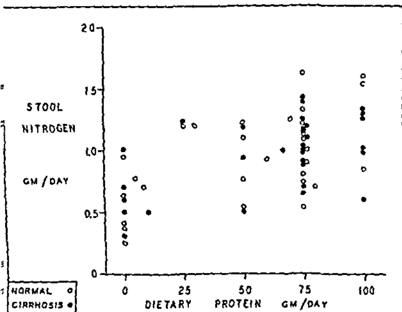


Figure 1 Stool nitrogen output in patients with cirrhosis of the liver and in normal subjects at various levels of dietary protein intake

of protein daily excreted from 0 7 to 1 4 gm of fecal nitrogen daily the normal subjects from 0 5 to 1 6 gm daily ranges of values which are comparable in the two groups The analyses of feces for nitrogen were made on from one to five

of absorption and metabolism of protein in severe cirrhosis[1]

During studies designed primarily to elucidate the role of dietary factors on the course of cirrhosis[2, 3] the urinary nitrogen excretion was determined during periods in which patients ingested food providing calories but essentially devoid of protein nitrogen The minimal urinary nitrogen excreted under these circumstances provided evidence concerning the rate of breakdown of endogenous protein These data are presented in TABLE 1 Three of the patients were given diets containing 0 7 to 2 1 gm of nitrogen and providing 2500 to 3500 calories daily as carbohydrate and fat These patients excreted from 2 3 gm to 4 7 gm of urinary nitrogen daily Stool nitrogen excretion was about 0 6 gm. daily Four other patients receiving 1600 calories as glucose solution orally and from 0 2 to 0 3 gm of nitrogen daily excreted 2 1 to 4 1 gm of urinary nitrogen daily These values for urinary nitrogen excretion under conditions of minimal nitrogen intake indicate that the catabolism of

TABLE 1
CIRRHOSIS OF LIVER MINIMAL NITROGEN EXCRETION

Patient	Days of study	Calories daily	N trogen gm per day (average)			
			Intake	Urine	Stool	Balan
J S	7	3500*	2 1	4 7	0 5	−11
M P	11	2500*	0 3	2 7	0 7	31
F C	13	2500*	0 7‡	2 3	0 6	51
J Mc	10	1600**	0 3***	2 9	0 6†	32
H C	4	1600**	0 2***	2 1	0 6†	23
S C	5	1600**	0 3***	2 5	0 6†	−28
W S	5	1600**	0 0	4 1	0 6†	47

* Fat and carbohydrate
** Glucose
‡ The n trogen conta ned n chol ne chlor de g en orally dur ng days 3 to 10 inclusive is include value
*** Th s n trogen was conta ned in chol ne d hydrogen c trate g ven orally da ly
† Est mated

'endogenous' nitrogen was not accelerated Further, the ingestion of 40 grams of glucose (20 per cent solution in water or in saline) provided su calories to prevent the utilization of significant quantities of nitrogen to the energy requirement under the conditions of the study

The data to follow concern the utilization of dietary protein by patients w cirrhosis of the liver Although many studies indicate that patients can

can be attained with moderate intakes of dietary protein sugges s tha patients are able to utilize dietary protein and that they are protein depl d All patients ingesting four grams or less of nitrogen daily are in negative nitro gen balance All patients receiving 12 gm or more of nitrogen da ly are positive nitrogen balance Most patients given 8 gm of nitrogen daily a in positive nitrogen balance It might therefore be conservatively sta that most patients with cirrhosis uncomplicated by other disease will mainta nitrogen balance if provided with daily diets containing 12 gm of nitrogen (gm of protein) and adequate calories These data indicate that even the e ceptional patients with the highest requirement for protein would mainta nitrogen equilibrium if given about 9 gm of nitrogen daily (56 gm of prote although most patients require less than this amount

That protein intakes of 75 gm daily will maintain positive nitrogen balan in patients with severe liver disease is further indicated by nitrogen balan

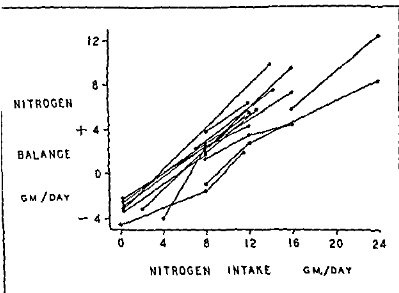


FIGURE 2 Protein requirement of patients with cirrhosis of the liver for maintenance of nitrogen balance

data obtained by the study of 16 additional patients with ascites who were given diets providing adequate calories and minimal intakes of sodium for study periods of from 15 to 30 days each A summary of the data obtained from these studies is presented in TABLE 2 Nitrogen balances in this group of patients ranged from +1 6 to +6 8 gm daily, and averaged +3 8 gm. daily, none being in negative balance under the conditions of study Thus, the protein requirement of these patients for the maintainance of nitrogen equilibrium or positive nitrogen balance may be greater than for normal individuals but is low enough to insure positive nitrogen balance when 75 gm (or even 56 gm) of protein is given daily when adequate calories are also provided "High" protein intakes were not necessary to achieve a positive balance

The data presented do not indicate that there is maximally efficient utilization of dietary protein by patients with liver disease Comparison of these patients to equally undernourished individuals without liver disease with regard to the efficiency of utilization of protein awaits some means of adequately evaluating an individual's state of protein nutrition Our clinical impression has been that, nutritionally it is more difficult to rehabilitate patients with chronic cirrhosis of the liver than patients who are undernourished due to starvation The problem, however has been approached in another manner Selected patients with evidence of severe active liver disease were maintained on constants diets, and urinary nitrogen excretion was followed for 40 to 50 days during which progressive improvement, as judged clinically and by laboratory tests, was manifest A progressive decrease in the urinary excre

TABLE 2

NITROGEN BALANCE SIXTEEN PATIENTS WITH CIRRHOSIS WITH ASCITES, 15–30 DAYS PER PATIENT, 2500–3500 CALORIES PER DAY

	N trogen gm per day			
	D et	Urine	Stool	Balance
Range	10 8–13 8	4 5–8 9	1 0–1 5	+1 6–6 8
Average	12 3	7 5	1 0	+3 8

tion of nitrogen was evident The data obtained from the study of two patients in this manner are presented in FIGURE 3 These studies suggest some defect in the utilization of dietary protein in the presence of active disease and a more efficient utilization with improvement

2 *Amino Acids*

The plasma concentration and the daily urinary excretion of alpha amino nitrogen were normal (TABLE 3), and the quantity of each of the ten "essential" amino acids excreted in the urine by patients with cirrhosis of the liver has been found not to differ markedly from that observed in normal individuals maintained on comparable diets[4, 5] The most marked deviations from the normal noted were an increased excretion of methionine and of tryptophan and a decreased excretion of isoleucine The increased excretion of these two essential amino acids was not considered to be nutritionally *limiting nor to alter* the patients' requirements for them This is substantiated by the failure of dietary supplements of methionine (and choline) to enhance nitrogen balance in patients with hepatic cirrhosis[6] However, the failure to observe gross deviations from the normal pattern of urinary amino acid excretion does not imply that the intermediary metabolism of each of the amino acids is entirely normal

In order to define further any abnormality in the urinary excretion of amino acids by patients with cirrhosis two additional methods of study were employed[5] One of these, designed to determine the effect of increases in dietary protein intake upon the urinary excretion of amino acids demonstrated that increases in protein intake did not result in the urinary wastage of amino acids During the other the effect of the severity of the liver disease upon the urinary excretion of the ten 'essential' amino acids was noted by observing the alterations in their urinary excretion which occurred concomitant with clinical improvement in patients provided with adequate and constant diets. Progressive decreases in the urinary excretion of these amino acids were noted, suggesting a more efficient utilization of amino acids with improved liver function The conclusion drawn was that the activity of the patient's disease influenced the urinary excretion of these amino acids more than did alterations in the dietary protein intake These data indicating a decreasing urinary excretion of amino acids with clinical improvement, and the studies mentioned above which indicate a decreasing urinary excretion of nitrogen under similar conditions of study suggest the presence in patients with severe liver disease of an

TABLE 3

PLASMA AND URINE ALPHA AMINO NITROGEN

	Patients with cirrhosis	Normal subjects
Plasma, mgm /100 ml		
No pts	12	35
Range	2 7–4 6	2 9–4 5
Average	3 9	3 7
Urine, mgm /24 hr		
No pts	15	10
Range	84–258	118–199
Average	171	152

TABLE 4

PLASMA ALPHA AMINO NITROGEN (MG PER 100 ML) FOLLOWING INTRAVENOUS INFU[illegible] 300 ML 15% PROTEIN HYDROLYSATE

	Before infusion	After infusion	
		1 hr	4 h
Patients with cirrhosis (8)			
Range	2 7–4 3	6 3–12 6	3 4–8 1
Mean	3 7	8 3	5 5
Normal subjects (5)			
Range	3 5–4 5	5 0–9 6	4 2–4 9
Mean	4 0	7 5	4 5

or other digestive disturbances may require the provision of nutrients by par-teral feeding Since the patients with damaged livers might not metabol- amino acids normally when given parenterally, the advisability of adminis- amino acids to these patients has been questioned For these reasons stud- were undertaken to evaluate the tolerance, excretion, and therapeutic value the intravenous administration of solutions of amino acids or protein hydro- sates to patients with cirrhosis of the liver Their tolerance to the rapid in- travenous infusion of a protein hydrolysate (300 cc, 15 per cent solution) not grossly abnormal (TABLE 4) The clearance of amino acids from the blo- by the patients was only slightly delayed as compared to normal individu- Furthermore, in one study, a progressive rise in serum alpha amino nitro- concentration did not occur in a patient receiving 100 gm of amino acids travenously daily for 18 days [8]

Further evidence that intravenously administered amino acids are [illegible] tabolized comes from the nitrogen balance studies over 2 to 4 weeks of [illegible] patients with active cirrhosis of the liver, given a solution of amino acids travenously daily as the sole source of nitrogen, while they were given a d[illegible] [8] Each of th[illegible] d with adequ[illegible] ia did not occ[illegible]

In addition improvement in the liver disease, as evidenced clinically and laboratory tests of liver functions, was observed during this period. T[illegible] pattern of the infused amino acids which were excreted into the urine by

that patients with liver disease are not able to remove ammonia adeq from the blood due, for example, to possible defects in the intermed tabolism of glutamic acid

Thus, although the mechanism of the reaction noted in the patients cirrhosis of the liver given the nitrogenous substances is not clear the s suggest that the administration of these materials may be harmful a offer additional evidence that a defect in nitrogen metabolism exists.

All patients given nitrogenous substances did not develop the unt reaction and, although all patients studied presented evidence of sever disease, whether or not a patient had a reaction did not relate to liver fu as measured by the usual tests For example, only two of six patients in the dietary protein intake was increased to 150 gm daily developed unt symptoms[10] Subsequent to this study, three additional selected patient been observed who displayed neurological signs attributable to incre dietary protein intake Even this incidence of reactions following d protein is significant, since large quantities of dietary protein are freq recommended in treatment On the basis of the observations cited, t visability of providing large quantities of nitrogenous substances inc dietary protein to patients with severe liver disease, especially thos hepatic coma may be questioned

Summary

Data have been presented which demonstrate that the absorption of n was normal in 28 patients with cirrhosis of the liver Studies of urinary gen excretion in seven patients given 1600 to 3500 calories and diets pro minimal intakes of nitrogen indicate that 'endogenous" protein was t tabolized at an accelerated rate Each of 32 patients studied demon positive nitrogen balance when provided with 75 gm of protein dai fact, most of these patients maintained positive nitrogen balance, whe vided with 50 gm of protein daily in a calorically adequate diet Fc ditional patients also maintained positive nitrogen balance when given ad quantities of amino acid solutions intravenously as a sole source of ni 'High" protein intakes were not necessary for patients with well-esta cirrhosis of the liver to achieve positive nitrogen balance

The urinary excretion of 'essential' amino acids by patients with c did not deviate grossly in pattern or in quantity from that observed in individuals given comparable diets or intravenous infusions of protein l ysate The urinary excretion of amino acids was affected more by the s of the liver disease than by the dietary protein intake Following th intravenous infusion of amino acid solution their clearance from the was only slightly delayed in the patients These data do not imply t intermediary metabolism of each of the amino acids or of the nones amino acids is normal in patients with liver disease

A defect in the utilization of ingested protein is suggested by the son greater protein requirement for the maintenance of nitrogen balance o of the patients than that for normal individuals by the decreases in nitrogen and amino acid excretion observed with clinical improveme

-nally, by the untoward reactions noted following the administration of nitrog- -nous substances including dietary protein

The studies presented do not indicate to what extent the dietary protein [illegible]

with this disease

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TREATMENT OF INFANTILE CIRRHOSIS OF THE LIVER WITH ANTIBIOTICS

By P. Krishna Rao

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Infantile cirrhosis of the liver is a peculiar condition found in Indian chi The disease commences insidiously between the ages of 6 and 18 months, which period the liver begins to enlarge gradually up to a size of four fingers' breadth with a comparatively small increase in the size In the last stages of the disease, there may or may not be jaundice, but is an invariable sequence At the final stages, death is due to hemorrhage

In my previous publication (1941), it was pointed out that the pat of infantile cirrhosis is that of Laennec's (portal) type and that the e falls into two stages It is my belief that, in the early stage, there is of the liver,

that, subsec

by *Escheric*

authors has discussed the view that lack of vitamin B factors, especially in food, may be involved in producing cirrhosis of the liver Bickn Prescott have said that milk is not a good source of choline (1946) *

In my previous paper (1951) on the treatment of infantile cirrhosis liver, it was shown that oral administration of choline and injections of tomycin have proved successful The object of the present paper is to briefly a further note on Streptomycin and the effects of other antibi the treatment of cirrhosis of liver

Streptomycin Many more cases have been treated with this drug sin and the results have been uniformly good. In fact, this has become the treatment for infantile cirrhosis cases in this part of India The usual c 4 to 5 gm, injected in daily doses of ¼ to ½ gm, continued over a pe 15 to 20 days

Aureomycin Four cases were treated with this antibiotic. Aure spersoids were used throughout One teaspoonful three times a day, g total quantity of 150 mgm. of Aureomycin HCl was the routine dose fo cases, and advanced cases received 250 mgm. or more per day Diarrh the usual complication, and the drug had to be stopped in the middle treatment for one, two, or even three days, until the diarrhea was con All the children died Therefore, this drug has not proved useful in the ment of infantile cirrhosis of the liver

Terramycin The next antibiotic being tried is Terramycin. This in the form of drops, the powder being dissolved in the solvent supplied manufacturers A daily dose of 600 mgm is administered in three

* Work is being carried out here in collaboration with Sri M Bhimsena Rao a Fellow of Research Board (Bombay) and Doctor K. P Basu at the Indian Dairy Research Institute, Bangalo choline contents of various milks human as well as other animals and also of the mothers of cirrhotic The results will be published in course of time

loses Six cases have been tried so far for the last six months Marvelous esults have been observed. In the case of four children with an early stage of liver enlargement (about 2 fingers), the liver came to normal size by the time ? gm of the medicine were completed Tonics containing choline methionine and vitamin B complex were given simultaneously as adjuvants Even in two other cases which were advanced with jaundice and ascites the results were

Remarks

I am aware of the fact that the number of cases treated is very small to draw any definite conclusions In accordance with my theory that *E coli* is responsible for bringing about the cirrhotic changes in the liver I tried all the various antibiotics that have been proved to act against this bacteria

cirrhosis and as such a trial should be carefully conducted and reported

Summary

In this series of cases in addition to Streptomycin two new antibiotics Aureomycin and Terramycin have been tried in cases of infantile cirrhosis of the liver Terramycin has been found to be very efficacious in curing the condition

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METABOLIC AND NUTRITIONAL PATTERNS IN ALCOHOLISM

By Jorge Mardones

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The reasons for considering the nutritional and metabolic patterns in alcohol addicts as differentiated from those of nonalcoholic persons are two the one based on clinical and the other on experimental viewpoints The clinical reason is that certain deficiency diseases appear with significantly greater frequency in alcohol addicts than in nonalcoholics These are liver cirrhosis, polineuropathy and other forms of thiamin deficiency, and pellagra The experimental reason is that it has been observed in rats and mice that when their diet lacks certain B complex vitamins they increase their voluntary ethyl alcohol intake, thus showing that nutritional factors can alter the appetite for alcohol

In human beings, the nutritional conditions depend, on the one hand, on the characteristics of the appetite, and on the other, on the ability to sat... it. I use the term "appetite" not in the restricted sense of the desire to ... food in general, but to eat certain foods

Human appetite has a very complicated mechanism, and it differs in each person Fundamentally, differences in the appetite are the result of genetic constitution, as well as of characteristics acquired by experience, education and pathological events In their daily fluctuation, they are the result of physiological changes and pharmacological actions

Thus, if a group of persons shows a nutritional pattern, it means that there are, in the constituents of the group, certain similitudes in the factors affecting nutritional conditions, namely, genetic constitution, experience, educa... ... the appe...

... m ...

exist in the social classes of low economic level in certain countries in which common factor of limited availability of foods is present Obviously, examples of this nature can be multiplied

In the case that concerns us now, however, in which we assume the presence of a nutritional pattern in a group, not as the result of a dietary survey but because of the frequency of deprivation diseases, the problem increases its difficulties because of the appearance of new variables In fact, the individual variations are now playing a new role since, with the same deficient diet different individuals show differences in the development of pathological consequences Such individual differences are produced particularly by genetic constitution and also by the occurrence of pathological conditions

All the preceding considerations force us to conclude that, if thiamin deprivation symptoms are frequent in alcohol addicts, it means that the addiction leads the patient to consume a diet low in thiamin or that a relationship of some other nature exists between addiction and thiamin deprivation In fact, it is possible that alcohol addicts are more sensitive to thiamin deprivation

hat thiamin deprived persons are more likely to become alcohol addicts *Mutatis mutandis*, the same can be said concerning liver cirrhosis and low protein iet, as well as in the case of pellagra and nicotinic acid deficiency

Let us now consider each of these points There is no doubt that, during heir so called "benders," alcohol addicts reduce their food intake to undernutrition levels, and this may be the cause of deficiency diseases, but, on the ther hand, we have to consider that undernutrition is frequent enough in ertain populations so that a more even distribution of these diseases may be

Obviously one question must be posed first, namely, does alcohol ingestion ncrease the sensitivity of individuals to these deficiency diseases?

Concerning thiamin, it has been established by Lowry, Sebrell, Daft, and

therefore, too soon to review this problem In any case, it has not been proved

the development of these deficiency diseases, it is not the direct effect of the

that, at the same time, favor both the development of alcohol addiction and the above mentioned diseases

There is no doubt that the psychological factors which play a role in the pathogenesis of alcohol addiction are similar to those which induce other forms of mental trouble It is therefore, necessary to assume that there are personal conditions favoring the influence of these psychological factors toward alcohol addiction

It is now clear why it is important to review the experimental facts th convince us that other kinds of relationships can exist between nutrition conditions and habitual drinking of alcohol

Richter and co workers, in papers appearing between 1937 and 1941 reported that when normal rats are free to choose between foods, they eat a balanced diet[15] and that they adapt the diet to the needs changed by experimental endocrine disturbances[14, 16, 19] or by vitamin deficiencies[17] Richter and Campbell[18] on the other hand, reported that, under free choice, rats prefer a weak alcohol solution to tap water Mardones and Onfray, in 1942,[8] having in mind the idea that a disturbance of the carbohydrate metabolism between C_3 and C_2 compounds could increase the desire for alcohol, studied, with rats the influence of a diet deprived of thiamin on the voluntary alcohol intake Their first showed that, in these circumstances, rats significantly increased the alcohol intake If these rats received a thiamin supplement, they improved their weight and general appearance but they maintained the high level alcoh intake If, however, they received a supplement of liver, meat, wheat germ untreated yeast, the voluntary alcohol intake rapidly reached the normal lev Since the diet lacking thiamin contained autoclaved yeast, we claimed th presence of a new thermolabile factor of the B complex which we designa factor N Subsequently my associates and I[10] reported that, if the suppleme of thiamin was maintained for some weeks, the rats decreased the volunta alcohol intake which reached the normal level in about twelve weeks, and th if normal rats were fed on a diet containing autoclaved yeast plus thiamin th voluntary alcohol intake was maintained at the normal level On the oth hand, rats fed a diet containing only ten pure vitamins of the B complex an showing a high level alcohol intake, decreased the alcohol intake in a sm way after supplementation of yeast, regardless of whether the yeast was au claved or not The thermostable factor present in yeast and liver responsib for the decrease of the alcohol intake in these conditions has been called fact N_1 Actually, these findings show that factor N is composed of thiam (thermolabile), and N_1 (thermostable)

In the meantime, Brady and Westerfeld[3] reported new facts along this lin These authors confirmed that rats fed on a diet deprived of all the elements the B complex rapidly increased the voluntary alcohol intake, but they observ that these rats decreased it only temporarily after each continuous supplemen of vitamins On the other hand, they reported that the same diet that pr vented the increase of the alcohol intake is unable to maintain it at a low lev in rats that have reached a sizable intake The authors suggested then th this fact means that "nonnutritional factors complicate the picture"

The facts reported by Brady and Westerfeld were explained later on Bee stecher and co workers[1] showed that a single deprivation of riboflavin, pyr doxine, or pantothenate also increased the voluntary alcohol intake of rats an that it dropped suddenly when the specific deprivation was corrected Th fact led to the interpretation that the transitory drop observed by Brady an Westerfeld was the result of partial satisfaction of the deficiencies that pr duced the increase of the alcohol intake On the other hand, we have reporte that, if a sufficient amount of yeast supplement is given to rats with high lev

ol intake, the decrease of the voluntary intake was permanent Thus, econd fact reported by Brady and Westerfeld is presumably the result of

e substance present in liver or yeast that can decrease the alcohol intake

nesium level in blood. No biochemical reason has been postulated to ain the relation between these findings and the excessive alcohol intake ecently Varela Penna Alcamo Johnson and Mardones[11] have initiated a ly of the carbohydrate metabolism in human alcohol addicts with the idea a disturbance in it could explain why the energy of the alcohol is more

available to alcoholics than that of the carbohydrates Their finding show that the glucose tolerance did not differ in the group of alcoho from that of social drinkers and abstainers but that some differences the two groups in the blood pyruvate curve and in the blood acet curve after glucose ingestion are significant at the 2 per cent level results cannot yet establish the presence of a metabolic distu alcohol addiction, but it encourages new stu lies along the same l ne

After this paper was prepared, I became aware of some new facts I wish to comment briefly as follows

Recently Lester, Greenberg Smith, and Wu[3] reported a finding tl feres with the interpretation of the voluntary alcohol intake in rat observed that, if rats showing high level alcohol intake were allowed sucrose or saccharin solutions they decreased significantly the alcoh It is not easy to interpret this fact since those rats had free access containing sucrose, which means that their appetite for this suga according as it was in solution or in crystalline form On the other l interpretation is complicated by the circumstances that the experim used showed a very high level alcohol intake, that the diet was low hydrate, and that the rats were not actually depleted In any case these results would necessitate study of the effect of nutritional imb the selection between an alcohol solution on the one hand and a suc tion on the other, instead of a dry diet containing crystalline sucrose

Two other facts have been reported that are related to the probl discussion The first one was published by T B Sirnes[40] of the De of Pharmacology of the University of Oslo He observed that rats liver cirrhosis was induced by treatment with carbon tetrachloride significantly their voluntary alcohol intake, and that this increase i sequence of the cirrhosis induced and not of other pharmacolog cal carbon tetrachloride This shows that a liver lesion can induce alcohol intake

The other fact is reported in this monograph by Olson and Du page 889) They observed that, in the liver of rats with dietary nec degeneration there is an important depression of the pyruvate oxida

All the facts analyzed in this talk induce us to believe that volunta intake is increased when a difficulty in the utilization of the energy hydrates or fat is present and that this difficulty does not interfer utilization of the energy of the alcohol They also induce us to sus in some cases the excessive ingestion of alcohol is not the cause bu sequence of nutritional or metabolic difficulties

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THE GENETOTROPHIC CONCEPT—NUTRITIONAL DEFICIENCIES AND ALCOHOLISM

By Roger J Williams

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A full appreciation of the general and far reaching significance of the trophic concept[1, 2] requires that we recast, if not revolutionize, our ideas regarding how we should use statistical methods in our attempts to solve biological and human problems

The revolutionary point of view which enters into this concept is in line with the current opinion of geneticists that every genotype determines its 'norm of reaction' to the environment[3] and that no species can adequately be described in terms of a type specimen It is also in accord with the idea of partial genetic blocks[4, 5] which recent evidence has indicated to be the rule rather than the exception[6]

In the areas of physiology psychology, biochemistry, and medicine, as well as in philosophy and the social sciences we have built into our thinking the concept of *the normal man* whom we regard as of paramount importance We have set it as our supreme task to understand how this hypothetical creature functions In the field of animal biology, the rat, the guinea pig or the salamander etc become the center of our attention This is the view which is incompatible with the genetotrophic concept

Biological variability is recognized by the biological fraternity as a more or less necessary evil When it is extreme, it makes very difficult the establishment of norms However by proper statistical treatment, its effects are smoothed out and we are as it were back on the right track again. The tacit assumption is made that the normal man is normal through and through, and that abnormalities are to be observed only in those who belong in the category of the abnormals This assumption is manifestly false, as will be made clear in our discussion Nevertheless it is commonly accepted and underlies the firmly intrenched idea of the normal man (or rat or guinea pig) which appears to be a guiding concept for most monographs advanced books and research publications in all of the areas of biological science

This attitude which centers its attention on those supposed individuals whose attributes always lie within the normal range is based in part upon another idea which is firmly intrenched in much biological thinking namely that on the microscopic level the cell is the center of our interest If we say we can ascertain how the cell is constructed and how it works we will have gone a long way toward understanding biology

The fundamental reason why we construct the normal man' in our minds is so we can develop *generalizations* which in a sense are the essence of science Scientific progress in the area of biology as well as in other areas may be measured in terms of the number and scope of valid generalizations which exist the more far reaching the generalizations the more advanced the science

It is my broad thesis however that biological variability in the human

inevitable, but those which are made without regard for human variability are very likely to be premature and completely misleading Leo Loeb,[7] who has devoted a lifetime of study to variability, has said that individuality is most highly developed in the human species, and this observation alone should make us alert to its possible implications

Let us look hastily at a number of human problems which naturally fall in the hitherto undeveloped field of *applied human biology* Consider sex crimes for example Can one be at all sure that "the normal human being" commits these, and that *deviates* in the area of sex physiology are not responsible? If we admit that this is an area where deviation may be important, how about the broader problem of sex adjustments in which all human beings are concerned? Can anyone say *a priori* that these adjustments characteristically involve "the normal human" and that the enormous variability which Kinsey's report brought to light (much of which is probably basically inborn) has nothing to do with these adjustments? Marriage and divorce, and hence our whole social structure, have deep biological roots, and it seems probable that the enormous variability in sex urges, inclinations, and predispositions are of paramount importance and that the very deviations (which may be found in any of us) merit serious study instead of being smoothed away by statistical techniques

I should like to interpose here another reason why "man" or "the normal human being" has in the past been so much the center of interest We should like to think that, at the beginning of life we have an equal start and that we are the makers of our own destinies If many of our problems arise out of our hereditarily controlled variation, we reach an "apparently gloomy conclusion as Professor Dunn[8] puts it, 'toward which all results of heredity seem to be driving us,' namely, that our shortcomings and hence our difficulties are foreordained and hence irremediable This conclusion is by no means a valid one, and the genetotrophic concept points the way very significantly as we shall see later, toward its demolition or at least its radical modification

Consider next, if you will, the broad problem of health as a field of applied human biology How many so called metabolic diseases have their roots in inborn metabolic peculiarities? We know for example that diabetes does and, on the basis of present evidence, it seems probable that other metabolic diseases

answerable question

If we concede that inborn variability may be important in connection with metabolic diseases what shall we say about infective diseases? Professor Gowen of Iowa State College has recently shown[9] by combining a genetic study of mice with a study of their responses when challenged with tuberculosis germs of varying virulence that the problem is three dimensional as is shown in FIGURE 1

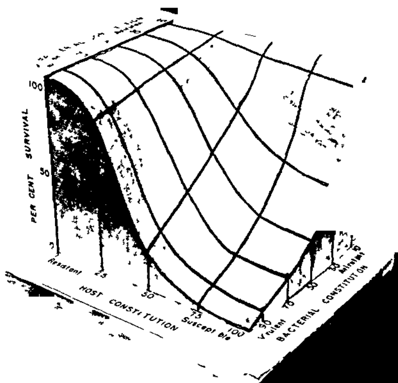


FIGURE 1

It would be difficult to controvert the idea that *all* infective diseases are three dimensional in the same way

There are numerous other diseases not classified as metabolic or infective for which the etiology has been for decades completely unknown or at least obscure Among these are cancer allergies mental disease, alcoholism dope addiction and many so called degenerative diseases

In all of these diseases a common fact may be observed The diseases attack only *certain individuals* while others in the same environment escape completely One member of a community may have cancer at a relatively early age Another, who has the same occupation eats the same food breathes the same air, drinks the same water lives in a similar house, is exposed to the same sunshine and weather may live to be 90 years of age with no threat of the disease A similar statement applies in the case of allergies Some people are unfortunate, others are fortunate One member of the community may develop mental disease, while his fellows who have perhaps just as many harassments and frustrations, remain well balanced Two individuals in the same environment can drink alcoholic liquors to the same extent over a period of

years One may, however, become an alcoholic, an uncontrolled drinker, while the other may remain a heavy but well-controlled drinker *When different individuals under hospital conditions are repeatedly given morphine, most of* them escape aftereffects, but some, for no known reason become addicts Heart disease is a type of degenerative disease which attacks certain individuals and not others, and there is apparently no environmental influence which determines who will be the victim

What hypothesis regarding these diseases can match for importance the one which postulates that in every case the individual's predispositions rest upon biological deviations from the respective norms? "Why is the 'normal human being' subject to allergy? Why does the 'normal human being' become an alcoholic? Why does the 'normal human being have heart disease?' These, from the standpoint of our discussion are foolish and misleading questions Far more in order are the questions 'What deviations do we find in those subject to cancer? What metabolic peculiarities make one subject to allergy? What is different about certain persons which makes them become alcoholics? What kinds of deviations do we find accompanying specific types of heart disease?'

It seems probable that learning about 'normal' characteristics is only a first step We must learn more and more about deviations and in order to do this, we must give them serious and attentive study We must not eliminate them by statistical treatment Instead we must search for them and discover their nature We must scrutinize them We must find out about their frequencies and be continuously aware of their existence and their possible influence I believe convincing evidence is actually at hand which shows that, mathematically and scientifically speaking no such thing exists as a human being who is normal through and through An individual may be normal'

[illegible]

ge, Kirk, Lewis, (1) *lipid phos*

phorus, (2) free cholesterol, and (3) lipid amino nitrogen From FIGURE 2 and the accompanying tabulated data in the original article it is evident that four individuals lie below the normal range (at the 95 per cent level) numbers 5, 18 29, 67, and one individual, number 63, lies above this range From FIGURE 3 and the accompanying data it is clear that there are two individuals below the normal, 51, 56, and three above 24 42 61 But these are not the same individuals that appeared abnormal in FIGURE 2 They constitute a completely new set of "abnormals" From FIGURE 4 and the accompanying data one individual number 13 is below the normal range and two numbers 35 and 51, are above it Here appears another new set of abnormals entirely different from the others

It does not take very much imagination to conclude that if these 67 indi

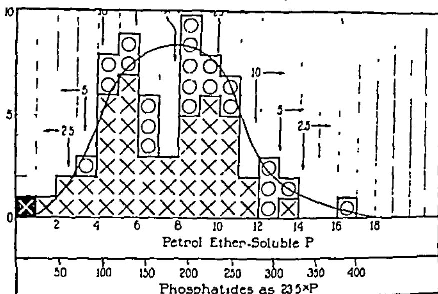


FIGURE 2

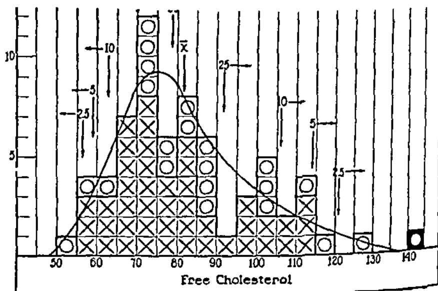


FIGURE 3

viduals were studied further and if the series of measurements were extended to include many diverse items, all of the 67 would be found to be "abnormal in some respect. A substantial number of deviations are probably present in the makeup of each of us. Deviation is universal and, if an individual should turn up who is in the "normal range" with respect to every item in his makeup, he would be a most extraordinary and, in fact, a most "abnormal" individual.

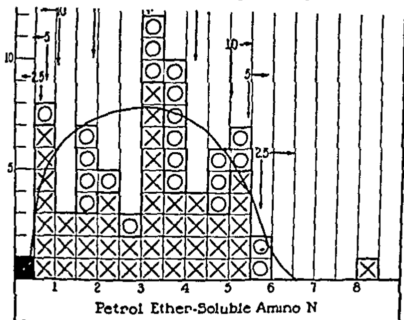


FIGURE 4

This point of view is further borne out by a consideration of the individual metabolic patterns[11] which we have studied at the University of Texas. Twelve individuals were measured several times with respect to 31 items 5 taste thresholds, 12 salivary constituents, 14 urinary items. FIGURE 5 is a drawing, using polar coordinates, representing the hypothetical case of an individual who is exaclty average with respect to every one of the 31 items. In FIGURES 6–17 are the corresponding diagrams for each of the twelve individuals using as the basis an average of the values obtained from each one.

In the pattern of *each* individual, there are from 2 to 5 items in which their distinctive values are one tenth or less that of the corresponding average values. In other words, the average, taken as a norm is of the wrong order of magnitude for some items in the case of *every individual in the group*. In this study, every finding points to the nonexistence of the human being who is "normal" in all respects.

In considering the effects of environmental influences on human beings—food composition, temperature light, infective agents, and drugs which may combat them, altitude, noise, housing and clothing, home and social influences, *etc.*—we are prone to think in terms of their effects upon the hypothetical "normal human being," and to disregard the biological variation which makes each of us a mosaic in which greater or lesser deviations are the rule rather than the exception.

In the field of nutrition, emphasis on the concept that people, as a rule, are

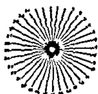


FIGURE 5

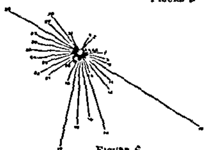


FIGURE 6

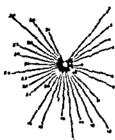


FIGURE 7

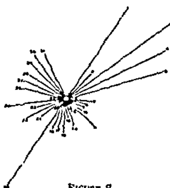


FIGURE 8

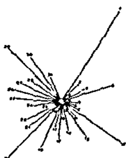


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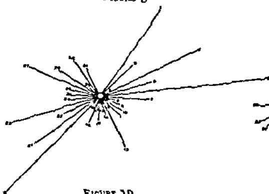


FIGURE 10

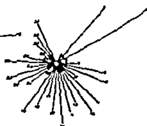


FIGURE 11

FIGURES 5 1? [illegible]
6 Uric acid ? [illegible]
15 Serine, 16 G [illegible]
21 Gonadotrop [illegible]
Creatinine 27 [illegible]

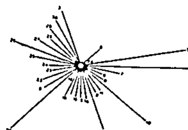


FIGURE 12

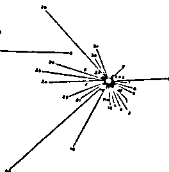


FIGURE 13

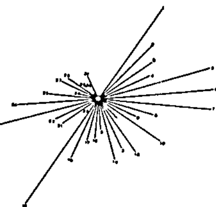


FIGURE 14

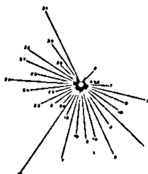


FIGURE 15

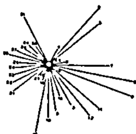


FIGURE 16

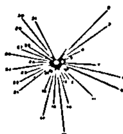


FIGURE 17

consistently normal is particularly pertinent Indeed, if the population consists essentially of normal human beings who have substantially identical nutritional needs, then the genetotrophic concept about which I am to speak is invalid and must be completely disregarded

In the light of modern biochemical genetics, however, this uniformity in nutritional needs cannot exist Genes in a sense beget enzymes, and enzymes require substrates in the form of foods and the metabolites derived from them Since the assortment of genes is distinctive for each of us, we must each have a distinctive pattern of enzymes and enzyme systems of varying efficiencies, and it follows, therefore, that our substrate needs are not the same, quantitatively speaking The widespread prevalence of partial genetic blocks[4,5] or their equivalent, makes it reasonable to suppose the nutritional needs of each of us for calcium, for leucine, for vitamin A, for riboflavin *etc*, are distinctive. The variabilities may be large or small, but they are inescapable There is reason, I believe, to suspect that the deviations are much larger than has traditionally been thought They certainly have not been studied sufficiently so they can be dismissed as inconsequential

With this discussion as a background, it becomes easy to indicate the meaning of the genetotrophic concept It is simply that some individuals because ... are likely

as a result If, however, such individuals are furnished an extra supply of the items which they individually need, their difficulties should disappear and their status should be as though the basic genetic peculiarities did not exist

We have postulated that numerous diseases of which the etiology is obscure may have genetotrophic origins[1,2] Such diseases by definition are of genetic origin but are also nutritional (trophic) in nature and hence ideally may be eliminated by nutritional means

A clear cut case where the genetotrophic concept appears to apply is the disease alcoholism which I wish to discuss briefly Actually, the genetotrophic concept grew out of our experimental findings while we were studying the problem of alcoholism and hence in this discussion I have in a sense placed the cart before the horse This has been done deliberately, however because the genetotrophic concept is much more far reaching in its implications than anything that could be said about alcoholism, and I hope that the subject of alcoholism will not crowd the broader aspect of the genetotrophic concept out of your thinking I do wish to make it clear however, that our theorizing ...ental find... ...st and

We did however after a careful survey of the literature, come to the conclusion that potential alcoholics probably have inborn differences which needed to be sought out[12]

Our first attack consisted in a prolonged and detailed study[3] of the metabolic patterns of a group of alcoholics who were not drinking at the time and of a corresponding control group The items investigated were more numerous,

TABLE 1
TABULATION OF HIGH AND LOW CHARACTERISTICS IN ALCOHOLICS IN ORDER OF DECREASING SIGNIFICANCE

High	Low
Sodium (S) (99)	
Hippuric acid (U) (99)	Gonadotrophin (U) (96)
Uric acid (U) (95)	Citrulline (U) (95)
Uric acid (S) (95)	
Thiamine (U) (94)	
Citrate (U) (92)	
Magnesium (B) (92)	
Pigment/creatinine (U) (91)	Phosphorus (B) (90)
Weak acids (U) (88)	Taurine (U) (88)
Sodium chloride (T) (88)	*Bacillus subtilis* (P) (88)

Numbers in parentheses are P values for difference between alcoholics and controls (S = saliva U = urine, B = blood serum T = taste threshold P = phagocytic index

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, we have several evidences bearing upon it Identical twins (FIGURES 16 and 17) were found to have patterns very much alike [11] indicating the

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all babies living largely on
Experimental animals ex-
highly distinctive excretion patterns when on identical diets [7] and an extensive study of several closely inbred strains of rats shows clearly that these patterns have a genetic origin [18] All these facts point to the conclusion that the alcoholics probably were deviates of the sorts indicated by their patterns *before* they began excess

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periments similar to this one had been done before by Richter[23, 24] and Mardones,[19] Brady and Westerfeld [25] and others The distinctive element in our study was that we ignored averages and concerned ourselves with the drinking behavior of *individual rats* We found a high degree of individuality Some rats drank considerable quantities of alcohol the first time it was offered and every day thereafter At the other extreme some rats were teetotalers One rat in the original group drank just a little alcohol every day as long as the

experiment lasted. Others drank a little at first and then after several weeks their consumption rose to moderate or high levels. Others increased their consumption to much higher levels in two or three weeks. In addition, some rats were spasmodic drinkers—they drank heavily for a day or two and then abstained for several days before indulging again.

Since these rats were on identical diets and their other environmental conditions were the same, the results suggested that heredity was an important factor in determining their individual drinking patterns. A study of other groups of rats, including six closely inbred strains (brother sister mated for many generations), has given ample evidence to confirm this conclusion. Rats from different sources show wide differences in their drinking patterns and especially in their responses to nutritional supplements. Most inbred strains of animals however, have not been bred for factors which cause uniformity in alcohol consumption and, hence, there is often wide intrastrain variation.

Further experiments confirmed the findings of Mardones and co-workers, as well as those of Brady and Westerfeld, namely, that dietary supplements decrease the alcohol consumption of rats. In fact, we found that the alcohol consumption of practically all the rats in our original colony could be brought to very low levels by administering the known vitamins, including vitamin B_{12}, and linseed oil as a source of unsturated fat acids, to animals on an otherwise standard diet. On a diet, marginal with respect to known B vitamins substantially all the rats would drink alcohol at a high level, but when the deficient nutrients were supplied, alcohol consumption often dropped dramatically overnight and would continue at a low or even zero level thereafter.

When the experiments were shifted to the use of another colony of rats of Wistar origin, exactly similar results were obtained, except that a much larger percentage of rats were "refractory" and continued alcohol consumption even after all known nutrients were supplied in what was thought to be abundant amounts. There was not the slightest doubt, however, about the dramatic response observed in the case of many rats.

It is desirable to point out here that no two laboratories could hope to duplicate each other's detailed findings unless they took extraordinary precautions to use rats of exactly the same genetic origin. Very wide divergences could easily result from strain differences in the animals. It may be well to emphasize, however, that inasmuch as similar results have been obtained hundreds of times, there is not the slightest doubt but that dietary deficiencies in experimental rats bring about greatly increased alcohol consumption, and that meeting these deficiencies (which is not always easy or even possible in all cases)

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riboflavin, pantothenic acid or pyridoxin are relatively effective in this respect, but that deficiencies of other nutrients, vitamin A, biotin, and choline are also somewhat effective, as judged both by the number of rats responding and the magnitude of the response. Studies previous to this one had shown that vitamin B_{12} is certainly a factor and that unsaturated fat acids probably are. It

which indicate that subjecting animals to flashing lights and continuous noise can increase alcohol consumption The most interesting experiments dealt with 'teetotalers' rats which on stock diets failed to drink alcohol. Some of

psychological stresses bring about physiological changes and it seems reasonable to suppose that the intricate stream of metabolism could be so changed by psychological stress as to augment the needs for specific nutrients Thus even when psychological factors are involved, the basic change may be metabolic and therefore subject to nutritional influence

answer is I believe a certain and unequivocal affirmative

It is needless to say that the gamut of problems which arise in connection with consumption of alcohol—economic, social, health—are not all to be solved by any single stroke The one problem with which we have been primarily concerned is *that of the drinker who cannot control his drinking* the man or woman who is seemingly forced by the first drink to take another and another until he or she becomes incapacitated for useful work It is our opinion and the findings support it that such craving is essentially physiological and very *often beyond intellectual control*

I have come in personal contact with several individuals, all of whom had a terrific craving for alcohol (in some cases of many years' duration) who have been transformed by the use of dietary supplements into individuals who could drink moderately without excess not for a period of days or weeks but actually for a year or more [30] The first individual was treated about three years ago and as long as he has continued the recommended dietary supplements he has been able to avoid drunkenness and, at the same time, regularly to drink beer, his favorite beverage

Treatment of alcoholism is complicated by the fact that, for some individuals, the enjoyment derived from drinking is so great that they would as soon part with their right arm as with this pleasure This is by no means true of all alcoholics *Some would gladly give up alcohol entirely* if the physical craving allowed them to do so People in general have to gain some pleasure from life otherwise they will not want to live and the alcoholic who looks to alcohol

book now in press [31] I have presented evidence which shows that every with similar training and similar educational leanings have extremely diverse 'want patterns and derive their satisfactions from life in entirely different ways The alcoholic is fortunate if he can cultivate reasons for living which do not include alcohol For such individuals I believe there is real hope for the future If nutritional supplements now available will not curb or obliterate

fashion I believe the case is also strong against the existence of individuals who are thoroughly well Certainly, the great majority of people that I know are unwell in one way or another, whether they consult physicians regularly or

the general population is concerned

In a great many cases, it seems reasonable to suppose, the widespread manifestations of unwellness, lack of vigor, lack of adjustment, failure to mature *etc*, are based upon the fact that each of us is a deviate in a number of different ways Some of the deviations which we possess may cause trouble and must *be understood before they can be managed* *Many of the deviations*, *however*, are an inexorable part of life and cannot and should not be dispensed with Even these desirable deviations, however, need to be understood if we are to understand ourselves

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Comments on the Paper

Doctor Daniel Feldman, *New York* The evaluation of any treatment regime directed against the disease, chronic alcoholism is fraught with many pitfalls The peculiar nature of the illness manifests itself in great variations in drinking patterns, and the personality of the chronic alcoholic is such that he may respond with a period of abstinence to almost any procedure used Unfortunately, these periods invariably end with a resumption of the drinking I think that, in order to evaluate the effectiveness of any therapy, the patient's previous drinking pattern must be clearly known and a sufficiently long time must elapse before any conclusions can be drawn I should think that a minimum period of observation of at least a year is necessary before an evaluation of the success of a therapy can be made

The very important question of inadvertent psychotherapy arises in any critical evaluation of a therapeutic regimen The part played by such relationships as suggestibility, support, and the magic value of the material used must be honestly assessed One cannot ignore the fact that because of his personality makeup the alcoholic is positively looking for such psychological aids As a matter of fact, it is extremely difficult if not virtually impossible for the person dispensing the medication to divorce himself from a psychological relationship of some sort Obviously this makes it impossible to eliminate this factor in the evaluation of any treatment regime aimed at curing or arresting the disease

Despite this however, I think that a therapy such as Dr Williams nutritional one can be honestly evaluated if certain things are borne in mind for example the use of the patient's past alcohol pattern as a control If a significant difference is observed between his drinking pattern before and after the nutritional regime, and if an equal amount of psychological help was given in the past then, one might suspect that the improvement noted might in some way be related to the use of the nutritional substances I am aware that this statement could be criticized and that it could be pointed out that the individual managing the nutritional therapy might because of his personality supply certain psychological aids that the alcoholic patient did not have before A controlled experiment making use of identical placebos and carried on for a sufficiently long period of time would be of major importance in determining the part played by nutritional treatment Because of the great number of variations in drinking patterns and because of the peculiar psychological makeup of the alcoholic, one must be extremely wary in interpreting the results of any particular form of therapy

As regards Dr Williams report on a patient who became a social drinker following nutritional therapy one can say that it certainly is an unusual result and it would be very important to follow this individual for a long period of time Not infrequently one sees alcoholics who can drink socially for periods of time but invariably revert to pathological drinking

Another point which is of great interest is Dr Williams deduction that al-

normal alcohol intake is associated with genetic nutritional abnormalities and, consequently, with nutritional deficiencies Why should alcohol, a substance which does not as far as we know, correct any of the nutritional deficiencies which may be present be selected by such individuals? The psychological effects of alcohol on the other hand, are well known and if one presumes that the alcoholic chooses this substance for its inhibiting effect on cerebral processes, one has at least a logical reason for its use The emotional disturbances and psychological difficulties of alcoholics are well known and most carefully studied data point to the fact that these difficulties have pre-existed the alcoholism itself I should like to know Dr Williams' feelings concerning these psychological difficulties and their relationships to the genetic–nutritional abnormalities which he postulates

I should like to summarize briefly our experience with Dr Williams's method of treatment

A group of 26 patients was treated at the outpatient clinic with nutritional supplements weekly interviews and in a good number of instances was attending A A as well The study was begun Oct 20 1952, but many of the group were not seen until several months later being added as they appeared at the clinic Fifteen patients in this group are still being seen with regularity and are not drinking as far as we know Ten of the group have failed to return and three are known to be drinking heavily Follow up of the remaining seven has as yet, been unsuccessful One patient is not attending the clinic because of job interference but reports by telephone When last heard from he was having no drinking problem and was continuing nutritional therapy Although there was loss of control this patient would not be considered even a moderately severe alcoholic

All of the 26 patients studied reported a sense of well being while on the nutritional regimen

A great deal of psychotherapeutic relationship was present in this group and our experience shows that it is practically impossible to avoid this If one cuts the patient off short a strong resentment and hurt to the imagined rebuff results and the patient almost invariably drops out However inasmuch as most of these patients had failed to control their drinking under similar supportive therapies in the past we were curious to see whether the inclusion of regimen to correct any nutritional deficiencies would make supportive therapy any more successful

Most of the ten failures occurred in patients who were under treatment longest and it may be that many of the 15 patients still in treatment have not been observed long enough for even the most preliminary of evaluation It may be significant that the ten failures occurred in so short a period as four months

Three patients were treated intensively at another clinic Treatment began in October 1952 with a potent nutritional supplement and visits were held down to the minimum necessary for accurate observation Such visits took place at three or four week intervals The supportive value of such visits became apparent from the patients own remarks

One of the patients was a steady pathological drinker whose inebriety was

interrupted by short periods of partial sobriety. He had followed this pattern

note that this patient stated quite frankly that the interviews were very helpful, and he would call for additional appointments between his regularly scheduled visits

The second patient was a severe periodic drinker, going on "benders" once or twice a year He abstained between bouts He reported an increased sense of well being during the nutritional regimen Four months after beginning treatment, he went on a severe prolonged drinking bout, exactly the same as those prior to therapy For two weeks prior to this, he reported that he could drink an occasional beer

The third patient is a compulsive periodic drinker with no control His only sobriety was attained through taking church pledges and, usually he anxiously awaited the end of such a period and promptly went on a bender The onset of nutritional therapy coincided with such a pledge He claims he generally feels better and although he thinks about drinking, there is no compulsion The pledge has not been in enforcement for two months now and the patient is still not drinking, the first time in his experience that this has happened Although he feels the "pills' have helped he states that the fact that he has had a physician at the clinic to whom to come has been a great help and relief to him, and he looks forward to his visits He has never before had any treatment

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NUTRITIONAL ASPECTS OF CIRRHOSIS IN ALCOHOLISM—EFFECT OF A PURIFIED DIET SUPPLEMENTED WITH CHOLINE*

By Gerald B Phillips† and Charles S Davidson

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The association of alcoholism and cirrhosis of the liver was observed well over a century ago and is now generally accepted That alcohol played an etiological role through a direct toxic action on the liver was the natural assumption to be derived from this observation until the demonstration that fatty cirrhosis similar to that seen in man could be produced in animals by means of low protein diets[1, 2] Moreover, attempts to produce cirrhosis in animals by the administration of alcohol supplements to an adequate diet have been for the most part unsuccessful[3] As a result, the concept of cirrhosis in the alcoholic has been reoriented toward the nutritional aspects, so much so, in fact, that many consider this disease simply as a nutritional deficiency

The evidence that cirrhosis in the alcoholic is a manifestation of a nutritional deficiency is not conclusive To be sure, the reports from various parts of the world which implicate malnutrition unassociated with alcoholism are impressive[4, 5] But most of the patients with fatty cirrhosis in this country are chronic alcoholics, and even though dietary intake is usually poor during periods of imbibition alcohol has not been exonerated as an important causative factor, although there is little positive evidence to incriminate it Another argument for a nutritional basis for the cirrhosis of the alcoholic stems from the therapeutic efficacy of the regimen proposed by Patek and Post[6, 7] which emphasizes a nutritious diet But the success of this regimen in alcoholics drinking up till the time of admission to the hospital in itself cannot be attributed to the resumption of an adequate diet, as withdrawal of alcohol and other influences incident to hospitalization are incurred simultaneously

Probably the most convincing evidence against an hepatotoxic action of alcohol has been the demonstration that the administration of alcohol in addition to a nutritious diet to chronic alcoholics with liver disease did not prevent hepatic functional and histological improvement in one patient[8] or exacerbate the liver disease of four patients already treated even though given for as long as 6 to 18 months[7] Whether the alcohol consumed by these patients was quantitatively and qualitatively the same as that ingested before hospitalization would not be ascertainable

Thus although liver disease is certainly related to alcoholism, it is still not clear whether the liver disease of the alcoholic is a result of alcohol poisoning nutritional deficiency, or both

The discrepancy in our knowledge of the nutritional aspects of cirrhosis in the laboratory animal as compared to man is comprehensible and is attributable

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† On assignment from the National Institute of Arthritis and Metabolic Diseases U S Public Health Service

largely to the greater limitations of clinical investigation As a result, we have found it necessary to approach the problem of cirrhosis in the alcoholic in a negative manner This entails a study of the effects of nutrients on the course of the liver disease and, admittedly, is limited in establishing cause

In order to determine the effect of a dietary regimen on the course of the liver disease it is almost mandatory to study a patient with active disease, so that definite changes in the hepatic disturbance, in one direction or the other, can be discerned over a relatively short period of time We have found the most useful indices of activity in the cirrhosis of the alcoholic to be the serum bilirubin and urine urobilinogen concentrations and prefer some elevation of both in the patients to be studied The criteria that we have generally used to evaluate the course of the liver disease fall into two main categories (1) hepatic function" tests, and (2) hepatic histology Measurement of hepatic size is so inaccurate that the small changes expected in short term studies might easily be missed

One of the principal difficulties in the evaluation of diets and dietary supplements in chronic alcoholics with active liver disease is the rapidity with which these patients usually improve, in our experience, following hospitalization and consumption of even meager diets For this reason, many studies dealing with the comparative therapeutic efficacy of diets and dietary supplements in such patients have been relatively unrewarding Moreover even if a supplementary nutrient does not appear to augment the therapeutic value of a given diet it is nevertheless quite possible that a deficiency of that nutrient was responsible for the liver disease Rather than compare the therapeutic value of various diets and dietary supplements therefore it is first imperative to dissociate the effects of diet from the effects of alcohol withdrawal and hospitalization on the course of the liver disease *i e*, to determine whether alcohol withdrawal and hospitalization in themselves might ameliorate the liver disease In this respect the rapid improvement referred to above is advantageous as it permits the short term studies necessitated by the intolerability of markedly deficient diets If it could be shown that alcoholics with active liver disease did not improve on a basic diet despite alcohol withdrawal and hospitalization but did improve on a more adequate diet, then it could be concluded that one or more constituents of the more adequate diet was responsible for the improvement Individual dietary factors could then be added to this basic deficient diet to evaluate the effect on the liver disease Any factor present in food which proves efficacious might seriously be considered to play an important etiological role in the genesis of the liver disease

This method of approach using deficient diets has been employed in this laboratory for several years The results of these investigations are reported herewith those published being summarized those unrecorded presented in more detail

In 1949 *Eckhardt, Faloon and Davidson*[9] studied the effects of a protein free diet supplemented with intravenous amino acids on the course of four chronic alcoholics with active cirrhosis Although the basic diet was essentially devoid of protein (0 1 gm of nitrogen daily) it was shown to be capable of maintaining nitrogen balance and body weight when 85 gm of purified casein were added

daily as the sole source of protein [10] It contained no source of the vitamin B complex except choline (30 to 100 mg daily) The four patients were provided with the basic diet plus a multiple vitamin capsule (containing vitamins A C D, B_1, B_2, niacin, B_6, and pantothenic acid) and a 500 to 1000 milliliter infusion of a ten per cent solution of amino acids daily The average daily caloric intake ranged from 1700 to 4500 calories While on this regimen for a period of from two to four weeks, all of the patients exhibited a progressive decline in serum bilirubin, two showed a decrease in the size of the liver and spleen and in the one patient who was biopsied serially, there was a decided improvement in hepatic histology In one of the patients, moreover, the liver disease improved despite negative nitrogen balance

In a subsequent study, Eckhardt *et al* [11] followed three similar patients for from 8 to 13 days on the same basic diet with the same added vitamins but without the supplementary amino acids Each of the patients showed an improvement in "liver function" tests and a decrease in liver size Liver biopsies performed at the start and finish of the protein free diet period revealed no apparent change in fat content (by histology) except in one patient who received added choline for six days (12 gm of choline chloride daily) These results clearly demonstrate that rapid improvement in the liver disease can occur in alcoholics while they are consuming a diet essentially devoid of protein. The possibility arises that this improvement was related either to the alcohol withdrawal and hospitalization, to the protein free diet, or to both It is noteworthy, however that the three patients described had stopped alcohol intake and increased food consumption for from 4 to 7 days before the study began Consequently another explanation for the improvement must be considered *i e*, that these patients may have received enough of one or more beneficial dietary factors during the period just prior to study to initiate and even perpetuate recovery

To obviate this difficulty Phillips Gabuzda and Davidson[12] studied three patients with active cirrhosis who were allegedly drinking up till the time of study which started at the time of admission to the hospital Evidence for this in two of the patients was the presence of Wernicke s syndrome The patients were given no sustenance after admission except for a purified diet consisting solely of solutions of glucose and minerals which was continued for from 8 to 10 days One patient received thiamine, and another thiamine plus multiple vitamins in addition to the diet In contradistinction to the findings of Eckhardt *et al* [11] none of these three patients showed significant improvement in hepatic function size or fat content (by histology) After institution of an adequate diet for a similar period, there was improvement in hepatic function in all three patients and a decrease in hepatic fat in two, the third patient not showing a significant diminution until the biopsy taken after two weeks of the diet The failure to improve on a purified diet with subsequent improvement on an adequate diet for a similar period implied that there was something in the adequate diet responsible for the amelioration of the liver disease These results would tend to relegate alcohol withdrawal and hospitalization to subordinate influences in this improvement The failure to improve on this purified diet moreover affords the opportunity to assess the efficacy of individual nu

trients added to this diet on the course of the liver disease On the basis of the animal studies, choline and methionine would be logical factors to try Studies of this sort using choline have been carried out and the results follow

Five chronic alcoholics with evidence of active liver disease, who were drinking heavily and eating poorly until the time of admission to the hospital, were followed on the Thorndike Metabolic Ward No complicating illnesses were encountered except in one patient (H C), who developed transient fever associated with a moderate degree of delirium tremens After admission measurements the patients received no sustenance other than the purified diet of glucose and saline in four cases and glucose solution only in the one patient (C W) with evidence of fluid retention While on the purified diet, which

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Following the purified diet period, a diet containing approximately 1600 calories, 50 gm of protein and about the same amount of salt and water daily as was in the purified diet was instituted in five patients In three of these

retention, serum bilirubin thymol turbidity and flocculation, and urine urobilinogen and bile are given in a previous report [12] Serum alkaline phosphatase† was measured by the method of Bodansky [13] serum total cholesterol‡ as described by Schoenheimer and Sperry [14] and by Abell *et al* [15] and serum phospholipid‡ according to Schmidt *et al* [16] Liver size was estimated by measuring the distance of the liver edge below the right costal margin in the midclavicular line on maximal inspiration Liver biopsies were performed on admission on the five patients and repeated serially on four All biopsies were done transthoracically using the Vim Silverman needle and were generally uneventful For serial biopsies, an attempt was made to insert the needle at the site of the previous puncture

The results on each patient will be presented separately

Case Studies

Case 1 Patient J M was a 51 year-old white male chronic alcoholic of 30 years duration who was hospitalized 10 to 12 years prior to admission for jaun

* Chol ne d hydrogen c trate (Abbott Laboratories North Chicago Ill nois)

† The authors are grateful to Doctor Stephen Maddock Surg cal Research Laboratory Boston City Hospital in whose laboratory the determ nations were carr ed out

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Following the purified diet period, a diet containing approximately 1600 calories, 50 gm of protein, and about the same amount of salt and water daily *as was in the purified diet*, was instituted in five patients *In three of these* the caloric and protein contents of the diet were raised to about 3000 calories and 75 gm, respectively, for a subsequent period

The measurements of hepatic function and size made on admission were repeated at appropriate intervals The methods for determing bromsulfalein retention, serum bilirubin, thymol turbidity and flocculation and urine urobilinogen and bile are given in a previous report [12] Serum alkaline phosphatase† was measured by the method of Bodansky [13] serum total cholesterol‡ as described by Schoenheimer and Sperry[14] and by Abell *et al* [15] and serum phospholipid‡ according to Schmidt *et al* [16] Liver size was estimated by measuring the distance of the liver edge below the right costal margin in the midclavicular line on maximal inspiration Liver biopsies were performed on admission on the five patients and repeated serially on four All biopsies were done transthoracically using the Vim Silverman needle and were generally uneventful For serial biopsies, an attempt was made to insert the needle at the site of the previous puncture

The results on each patient will be presented separately

Case Studies

Case I Patient J M was a 51-year-old white male chronic alcoholic of 30 years duration who was hospitalized 10 to 12 years prior to admission for jaun

* Choline dihydrogen citrate (Abbott Laboratories North Chicago Illinois

† The authors are grateful to Doctor Stephen Maddock Surgical Research Laboratory Boston City Hospital in whose laboratory the determinations were carried out

‡ The authors are grateful to Doctor David Hurwitz Chief of Diabetic Clinic Boston City Hospital, in whose laboratory the determinations were carried out

daily as the sole source of protein [10] It contained no source of the vitamin B complex except choline (30 to 100 mg daily) The four patients were provided with the basic diet plus a multiple vitamin capsule (containing vitamins A C D, B_1, B_2, niacin, B_6, and pantothenic acid) and a 500 to 1000 milliliter infusion of a ten per cent solution of amino acids daily The average daily caloric intake ranged from 1700 to 4500 calories While on this regimen for a period of from two to four weeks, all of the patients exhibited a progressive decline in serum bilirubin, two showed a decrease in the size of the liver and spleen and in the one patient who was biopsied serially, there was a decided improvement in hepatic histology In one of the patients, moreover, the liver disease improved despite negative nitrogen balance

In a subsequent study, Eckhardt *et al* [11] followed three similar patients for from 8 to 13 days on the same basic diet with the same added vitamins, but without the supplementary amino acids Each of the patients showed an improvement in "liver function" tests and a decrease in liver size Liver biopsies performed at the start and finish of the protein free diet period revealed

cur in alcoholics while they are consuming a diet essentially devoid of protein. The possibility arises that this improvement was related either to the alcohol withdrawal and hospitalization, to the protein free diet, or to both It is noteworthy, however, that the three patients described had stopped alcohol intake and increased food consumption for from 4 to 7 days before the study began Consequently, another explanation for the improvement must be considered, *i e*, that these patients may have received enough of one or more beneficial dietary factors during the period just prior to study to initiate and even perpetuate recovery

To obviate this difficulty, Phillips, Gabuzda, and Davidson[12] studied three patients with active cirrhosis who were allegedly drinking up till the time of study, which started at the time of admission to the hospital Evidence for this in two of the patients was the presence of Wernicke's syndrome The patients were given no sustenance after admission except for a purified diet, consisting solely of solutions of glucose and minerals, which was continued for from 8 to 10 days One patient received thiamine, and another thiamine plus multiple vitamins in addition to the diet In contradistinction to the findings of Eckhardt *et al* [11] none of these three patients showed significant improvement in hepatic function size, or fat content (by histology) After institution of an adequate diet for a similar period, there was improvement in hepatic function in all three patients and a decrease in hepatic fat in two, the third patient not showing a significant diminution until the biopsy taken after two weeks of the diet The failure to improve on a purified diet with subsequent improvement on an adequate diet for a similar period implied that there was something in the adequate diet responsible for the amelioration of the liver disease These results would tend to relegate alcohol withdrawal and hospitalization to subordinate influences in this improvement The failure to improve on this purified diet, moreover, affords the opportunity to assess the efficacy of individual nu

TABLE 1

CLINICAL AND LABORATORY DATA OF FIVE CHRONIC ALCOHOLICS WITH CIRRHOSIS OF THE LIVER WHO RECEIVED A PURIFIED DIET AND CHOLINE FOLLOWED BY A MORE ADEQUATE DIET FOR THE SAME PERIOD

All measurements were made at the end of the specified days of diet except the "Initial" measurements which were made on admission to the hospital

Patient	J M			F C			J D			C W			H C		
	Initial	Purified diet and choline†	1600 cal 50 gm prot diet	Initial	Purified diet and choline†	1600 cal 0 gm prot diet	Initial	Purified diet and choline†	1600 cal 50 gm prot diet	Initial	Purified diet and choline†	1600 cal 50 gm prot diet	Initial	Purified diet and choline†	1600 cal 50 gm prot diet and choline†
Days of diet		10	10		9	9		7	7		5	5		4	4
Bromsulfalein % retention	40	21	13	43	34	22	61	60	53		38	39	33	34	35
Serum bilirubin mgm/100 cc 1 min	1.5	0.40	0.25	1.3	0.67	0.25	5.2	6.1	3.4	1.7	2.5	1.7	4.5	5.8	6.7
Serum bilirubin mgm/100 cc Total	3.2	1.6	0 "4	2.5	1.4	0.74	10.8	11.3	6.8	3.7	3.1	3.7	9.6	12.0	13.8
Thymol turbidity*	>8.7	3.1	2.9	3.3	3.0	2.6	8.0	6.9	4.4	2.9	2.9	2.3	>8.7	>8.7	8.5
Thymol flocculation (0-4+)	0	0	0	1+	0	0	2+	2+	2+	0	0	0	3+	3+	3+
Urine urobilinogen	1:512	1:128	1:32	1:512	1:16	1:32	1:256	1:4	1:4	1:128	1:128	1:128	1:128	1:512	1:128
Urine bile (0-4+)	2+	0	0	1+	0	0	2+	3+	2+	3+	3+	±	4+	4+	4+
Serum alkaline phosphatase††	—	—	—	7.3	5.1	5.8	11.0	8.3	5.3	2.9	3.0	3.3	10.8	6.2	10.4
Serum cholesterol mg/100 cc	1041	519	363	184	182		3 8	327	327	83	74	75	615	513	501
Serum phospholipid mg/100 cc	835	3 8	265	141	149	—	532	316	330	32	35	31	508	531	560
Liver size** cm	14	12	11			—	7	8	8	5	7	6	11	11	11

† See text for quantities
* Maclagan units
†† Bodansky units
** Below costal margin in mid-clavicular line on maximal inspiration

dice, anorexia, and weakness, and was told at that time that he had a 'very slight cirrhosis" Two years before admission, his alcohol consumption was augmented and food intake concomitantly decreased For the three weeks before entry, he felt weak and anoretic, ate very little, and decreased his alcohol intake somewhat On admission, his liver edge was 14 cm below the right costal margin, sharp but not very firm, and only slightly tender There was no definite edema, ascites, icterus, splenomegaly, or spider telangiectasia

Results (TABLE 1, FIGURES 1 and 2) During the first ten days of hospitalization, the patient received the purified diet and 6 5 gm of choline dihydrogen citrate orally daily Over this period, the bromsulfalein retention, serum bilirubin, and urine urobilinogen, which were all abnormal on admission, decreased progressively The thymol turbidity, which was very high on admission, likewise declined considerably, but since the thymol flocculation was consistently negative, this change may have been related to the steady decrease in serum phospholipid and total cholesterol, which were also quite high on entry The liver edge was thought to recede 2 cm during this time Following the ten days of the purified diet, the patient received for the next 30 days the 1600 calorie-50 gm protein diet but no added choline The improvement in the above measurements continued so that after 30 days of this diet, those remaining abnormal were only a bromsulfalein retention of 7 per cent and a hepatomegaly to 8 cm

The admission liver biopsy showed a large amount of fat with minimal fibrosis There were occasional polymorphonuclear leucocytes but no intracellular hyalin was seen Many of the cells appeared frayed and the nuclei were small and dark Repeat biopsies done after both 7 and 10 days respectively, of the purified diet and choline disclosed a definite but not marked decrease in fat and many cells with two nuclei, suggesting regeneration The cells were less frayed and the nuclei were more normal in appearance A biopsy taken after 10 days of the 1600 calorie 50 gm protein diet revealed still less fat, and one taken after 30 days of this diet showed only a slight to moderate amount of fat many binucleated cells, and significantly more fibrous tissue than was apparent on the previous biopsies

Case 2 Patient S C was a 48-year old white male chronic alcoholic of 20 years duration who usually drank intermittently in boutss lating 2 to 3 months and separated by intervals of abstinence lasting 4 to 9 months He had been drinking steadily and eating little for 8 months prior to admission During the two weeks before admission, he became quite anoretic and consumed very little food but continued drinking He claimed that he took 1 to 2 multiple vitamin pills* daily for 1 to 2 months prior to admission No history of previous hepatic disturbance could be elicited The admission physical examination revealed facial telangiectases, palmar erythema, and an enlarged liver with a moderately firm tender edge felt about 15 cm below the right costal margin No definite edema ascites icterus splenomegaly, or spider telangiectasia was made out

* Unicaps (Upjohn) Each capsule contains Vitamin A 5000 U, Vitamin D 500 U, ascorbic acid 3 2 mgm thiamine hydrochloride 2 5 mgm riboflavin 2 5 mgm pyridoxine hydrochloride 0 5 mgm calcium pantothenate 5 mgm nicotinamide 20 mgm folic acid 0 25 mgm and Vitamin B 12 activity 1 µgm

Table I

Clinical and Laboratory Data of Five Chronic Alcoholics with Cirrhosis of the Liver Who Received a Purified Diet and Choline Followed by a More Adequate Diet for the Same Period

All measurements were made at the end of the specified days of diet except the "Initial" measurements which were made on admission to the hospital

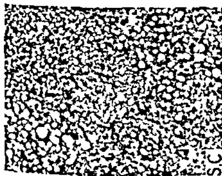
Patient	J M			S C			J D			C W			H C		
	Initial	Purified diet and choline‡	1600 cal 50 gm prot diet	Initial	Purified diet and choline‡	1600 cal 50 gm prot diet	Initial	Purified diet and choline‡	1600 cal 50 gm prot diet	Initial	Purified diet and choline‡	1600 cal 50 gm prot diet	Initial	Purified diet and choline‡	1600 cal 50 gm prot diet and choline‡
Days on diet		10	10		9	9		7	7		5	5		4	4
Bromsulfalein % retention	40	21	13	43	34	22	61	60	53		38	39	33	34	35
Serum bilirubin mgm/100 cc, 1 min	1.5	0.40	0.25	1.3	0.67	0.25	5.2	6.1	3.4	1.7	2.5	1.7	4.5	5.8	6.7
Serum bilirubin mgm/100 cc, Total	3.2	1.6	0.74	2.5	1.4	0.74	10.8	11.3	6.8	3.7	5.1	3.7	9.6	12.0	13.8
Thymol turbidity*	>8	3.1	2.9	3.3	3.0	2.6	8.0	6.9	4.4	2.9	2.9	2.3	>8.7	>8.7	8.5
Thymol flocculation (0-4+)	0	0	0	1+	0	0	2+	2+	2+	0	0	0	3+	3+	3+
Urine urobilinogen	1:512	1:128	1:32	1:512	1:16	1:32	1:256	1:4	1:4	1:128	1:128	1:128	1:128	1:512	1:128
Urine bile (0-4+)	2+	0	0	1+	0	0	2+	3+	2+	3+	3+	±	4+	4+	4+
Serum alkaline phosphatase††	—	—	—	7.3	5.1	5.8	11.0	8.3	5.3	2.9	3.0	3.3	10.8	6.2	10.4
Serum cholesterol mg/100 cc	1011	519	363	184	182	—	[illegible]	327	327	83	74	75	615	513	501
Serum phospholipid mg/100 cc	815	378	265	141	149	—	532	336	330	32	35	31	508	531	560
Liver size** cm	14	12	11			—		8	8	5	7	6	11	11	11

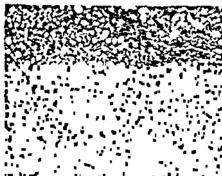
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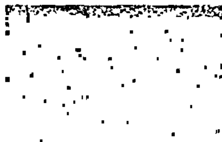
* Maclagan units

†† Bodansky units

** Below costal margin in midclavicular line on maximal inspiration







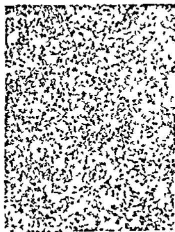


FIGURE 4. Biopsies for patient S.C. The row of biopsies is in chronological sequence from left to right. The first biopsy was performed on admission, the second after nine days of the purified diet and choline, and the third after nine subsequent days of the 1000 calorie 5 gram protein diet. The lower biopsy was obtained after twelve subsequent days of the 1000 calorie 5 gram protein diet. Hematoxylin and eosin stain. X40

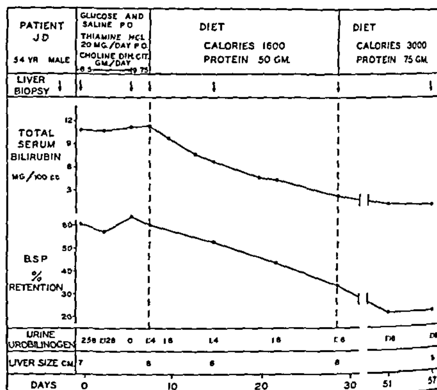


FIGURE 5.

Results (TABLE 1, FIGURE 5). This patient was provided with the purified diet for 7 days. In addition he received 6.5 gm. of oral choline dihydrogen citrate daily for the first 5 days, 9.75 gm. daily for the last two. 100 mgm. of thiamine hydrochloride orally on the first day, and 20 mg. orally daily over the next 6 days. On this regimen the serum bilirubin rose slightly but progressively and the bromsulfalein retention remained about the same, although the large amount of dye in the control specimens may have invalidated this measurement to some degree. The urine urobilinogen, however, dropped rather precipitously over this period and, at one time, was thought to be absent. Since stools were all brown this absence could not be attributed to intrahepatic block of bilirubin excretion. It is noteworthy that the patient had profuse watery diarrhea while on the purified diet and conceivable that this disturbance interfered with the production of urobilinogen.

For the next 3 weeks the patient was maintained on a 1600 calorie-50 gm. protein diet and for the four subsequent weeks on a 3000 calorie-75 gm. protein diet. After two days of the 1600 calorie 50 gm. protein diet, the serum bilirubin showed a significant decrease and after 7 days, the serum bilirubin showed a further decline along with a diminution in bromsulfalein retention. These tests continued to improve till the time of discharge. Liver size was difficult

to measure, as the patient's inability to relax frequently prevented detection of

morphonuclear leucocytes and no definite intracellular hyalin The cells appeared frazzled, and their borders were difficult to discern Many nuclei were small and dark. Repeat specimens obtained after 5 and 7 days respectively of the purified diet and choline displayed essentially the same abnormalities except for a suggestive decrease in fat and less frazzling of the cells After 7 days of the 1600 calorie-50 gm protein diet, there was a definite decrease in fat, but no other changes were detected except for an improved cellular architecture and an increase in the number of binucleated cells The biopsy taken after 3 weeks of this diet had much less fat and intracellular and canalicular bile Many binucleated cells were observed at this time The last biopsy specimen, which was secured after 4 weeks of the 3000 calorie 75 gm protein diet, contained only a minimal amount of fat but considerably more fibrous tissue than on the previous samples

Case 4 Patient C W was a 41 year-old white male chronic alcoholic of 25 years' duration who drank in bouts usually lasting a few months each with intervening periods of abstinence of about 3 months He had been drinking heavily and eating poorly for 7 months prior to admission He noted ankle swelling and diarrhea for about 5 days and increasing abdominal girth for 3 days before entry There was no past history of any of the stigmata of liver disease, but he did have ankle swelling 2 years before which disappeared when he stopped drinking Physical examination on admission disclosed a moderate amount of ascites and ankle edema and a firm smooth nontender liver edge made out with difficulty about 5 cm below the right costal margin No icterus splenomegaly, or spider telangiectasia was found

he purified diet plus
1600 calorie 50 gm
ein diet for 16 days

While he received the purified diet and choline the serum bilirubin rose and the urine urobilinogen remained the same No initial bromsulfalein test was done After only one day of the 1600 calorie 50 gm protein diet the serum bilirubin was significantly reduced and continued to decline over the next 19 days The bromsulfalein retention and urine urobilinogen however showed no change after 5 days of the 1600 calorie 50 gm protein diet and decreased slightly, if at all, during the remainder of the study The liver edge was difficult to palpate because of the ascites and the small changes noted are best disregarded

Only an admission liver biopsy was done This showed a moderate amount of fat and marked fibrosis and bile duct proliferation There were small numbers of polymorphonuclear leucocytes and scattered cells with typical intracellular hyalin

Case 5 Patient H C was a 44 year-old white male who drank in sprees for 30 years but steadily for the 2 years before entry About 4 months prior to admission he increased his alcohol intake and for 2 months prior to admission

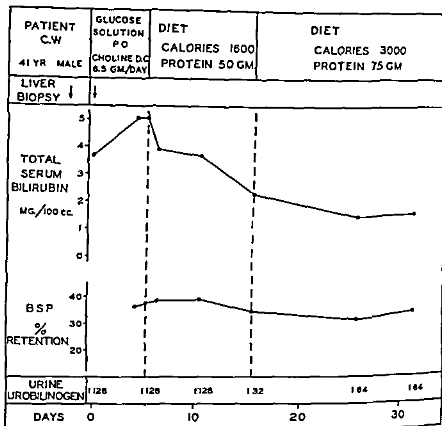


FIGURE 6

he was anoretic and ate very little He lost approximately 50 pounds in weight over the 3 to 4 months before entering the hospital Dark urine was noted for 3 months, weakness for 1 month, jaundice for 2 weeks, and light stools for 4 to 5 days before entry There was no previous history of any manifestations of liver disease Physical examination at the time of admission revealed a slight icteric tint to the skin with marked scleral icterus, a somewhat tender liver with a firm sharp edge extending 11 cm below the right costal margin, slight gynecomastia, and one spider telangiectasis No edema, ascites, or splenomegaly was made out

Results (TABLE 1, FIGURES 7 and 8) This patient was given the purified diet for 4 days, the 1600 calorie 50 gm protein diet for 10 days, the 3000 calorie-75 gm protein diet for 10 days, and the regular hospital diet for 28 days In addition, he received 3 25 gm of choline dihydrogen citrate daily for the first 24 hospital days After 4 days of the purified diet and choline, there was a rise in serum bilirubin and urine urobilinogen without a significant change in bromsulfalein retention After 4 subsequent days on the 1600 calorie 50 gm protein diet and choline, the serum bilirubin rose even further and the bromsulfalein

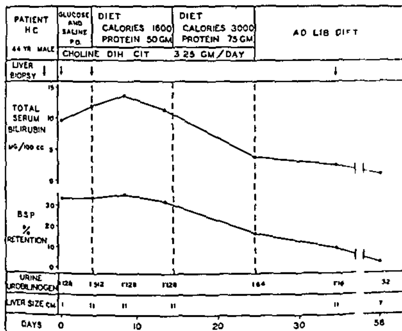


FIGURE 7

retention remained about the same, while the urine urobilinogen dropped. Six days later, however, the former two tests were decreased and, after that, all three tests gradually improved so that they were within normal limits after 52 days in the hospital. The thymol turbidity and flocculation showed no significant change over the first 14 days but were considerably reduced when measured 24 days after entry.

The initial liver biopsy revealed a small amount of fat with a great deal of intracellular and canalicular bile. There were occasional polymorphonuclear leucocytes and probably a minimal amount of intracellular hyalin. Fibrosis was slight in amount. A second biopsy performed after 4 days of the purified diet and choline showed no discernable change except for a possible slight increase in polymorphonuclear leucocytes. The biopsy taken about a month later, however, disclosed no fat but still an abundance of intracellular and canalicular bile. No intracellular hyalin or polymorphonuclear leucocytic infiltration was seen. In contrast to the first two biopsies, there was marked diffuse fibrosis.

Comment

Of five chronic alcoholics who were provided with the purified diet (glucose and saline) and choline orally for from 4 to 10 days, the active cirrhosis seemed

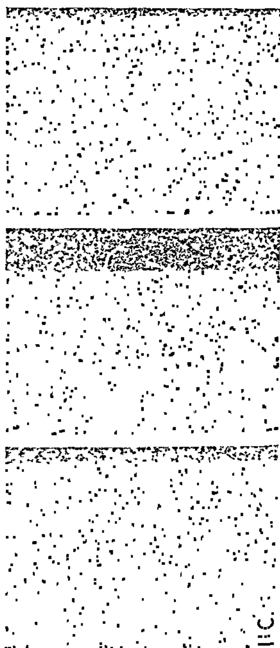


FIGURE 8. Biopsies for patient II C. The biopsies are in chronological sequence from left to right. The first biopsy was performed on admission, the second, after four days of the purified diet and choline, and the third after thirty subsequent days of increasingly more adequate diets. Hematoxylin and eosin stain. X50

to have improved in 2 and worsened in 3. Although it is possible that the latter three patients received a subtherapeutic quantity of choline, two of them consumed as much or more choline per day as did the two patients who improved. Insufficient gastrointestinal absorption may also have occurred since two of the patients whose liver disease became worse had diarrhea. Another factor to consider in one of these three patients was a complicating episode of delirium tremens, which may have contributed to the augmentation of the hepatic disturbance. When these three patients were subsequently provided with a more adequate diet, however, the liver disease improved, two of the patients showing a significant decrease in serum bilirubin within one and two days, respectively, after the diet was instituted. This failure to improve on the purified diet and choline with subsequent improvement on a more adequate diet implies that one or more constituents of the more adequate diet was responsible for the mitigation of the active hepatic disturbance and suggests, in turn, that a deficiency of the same one or more factors was important in the development of the liver disease. These results corroborate the findings in a previous study[1] in which the circumstances were similar except that the purified diet was not supplemented with choline. At least in these three patients, the factor does not appear to be choline alone in the amounts given over these brief periods of study. Neither is the factor just calories, as the more adequate diet was isocaloric with the purified diet. What the therapeutic component of the more adequate diet was, therefore, remains unknown.

Even though three of these patients, despite alcohol withdrawal, did not improve until provided with a more adequate diet, the alcohol may still have been an important factor in the development of their liver disease. Its role in this respect might have been to (1) accentuate a nutritional deficiency by

being more deleterious to the hepatic cell conditioned by malnutrition.

Improvement in the active cirrhosis did occur in two of the five patients during the period of the purified diet and choline. That choline may have been the therapeutic agent is suggested by the results of the previous study in which the active cirrhosis of three chronic alcoholics did not improve on the purified diet without choline but did improve rapidly on an adequate diet. Lack of adequate control studies, however, prevents this conclusion, as it is quite possible that the liver disease of the patients in the previous study was related to different etiological factors. Indeed, evidence that more than one important factor may be involved in the treatment and possibly the development of the liver disease of alcoholics is provided in the marked difference of response of the liver disease among the five patients in the present study while on essentially the same regimens.

Qualitative differences in the liver disease of the patients who responded favorably and those who did not may account for this discrepancy in response but are difficult to define. With respect to liver function, the only consistent difference was the higher serum bilirubin in the three patients whose liver function grew worse during the purified diet and choline period. With regard

hepatic histology, which was difficult to assess, the two patients who improved during the purified diet and choline period had a larger amount of hepatic fat than the others In fact, in the one patient who improved the more dramatically, the marked degree of fatty change was essentially the only hepatic lesion noted The hepatic fat of these two patients, moreover, appeared to decrease during the purified diet and choline period, but a similar change was also noted in one of the patients whose liver disease simultaneously became worse Thus, hepatic function did not appear to be related only to the fatty lesion, and other elements of the pathological picture, as fibrosis, intracellular hyalin, and necrosis require consideration In support of the view that fat is not the only histological lesion to consider was the marked acute hepatic dysfunction present in one patient whose liver biopsy showed only small quantities of fat It is possible that the response of these patients to various dietary regimens is related to the type of hepatic histological abnormalities present, but the small number of patients in this group forbids any rational attempt at correlation

One striking feature of the histological studies, which were done serially in four patients, was the apparent increase in fibrosis associated with recovery Although this change may have resulted from the disappearance of fat and condensation of the remaining tissue, the marked increase in fibrous tissue argued for a genuine progression of the fibrosis To support this impression was a decided increase in fibrosis seen in one of the patients who initially had only a minimal amount of fat Although the development of fibrosis could not be related positively to any histological feature, there were two bits of evidence against its relation to fat (1) The patient with the most hepatic fat developed the least fibrosis, and (2) the patient with minimal fat developed pronounced fibrosis What gave rise to the fibrosis, if real, therefore, cannot be stated from these studies

Although not all of the active cirrhosis of chronic alcoholics may be on the same etiological basis, in the great majority of these patients in our experience this active hepatic disease will improve rapidly following hospitalization of the patient and consumption of even meager diets In the present study, the active cirrhosis improved in two patients who received merely glucose saline and choline and in three patients who were given only 1600 calories and 50 gm of protein daily From our clinical studies, therefore we believe that effective dietary therapy for the active cirrhosis of the alcoholic is the withdrawal of alcohol and the provision of an adequate diet

Summary and Conclusions

(1) In a previous study it was shown that the active cirrhosis of three chronic alcoholics failed to improve when these patients were provided with a purified diet (glucose and minerals) but subsequently improved when given an adequate diet for a similar period These results suggest that one or more constituents of the adequate diet was responsible for this improvement

(2) In the present study five similar patients were similarly followed except for the addition of choline to the purified diet In three of the patients choline did not appear to be therapeutic In the other two patients, choline may have had a beneficial effect

(3) It is concluded that choline alone in the amounts and for the dura o given is not therapeutic for the active liver disease of all alcoholics Whe her choline is of definite therapeutic benefit in some cannot be conclud r m l c data presented

(4) The marked difference in response of these patients to in ar suggests that the liver disease of the alcoholic may be related to m re etiological factor

Acknowledgments

The authors are grateful to Dr G Kenneth Mallory the Ma r l of Pathology Boston City Hospital for reviewing the histolom l s

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Discussion of the Paper

DOCTOR HAROLD BLUMBERG, ENDO PRODUCTS INC, NEW YORK This investigation by Doctor Davidson and Doctor Phillips is certainly very interesting and valuable Theoretically, of course, the value of choline in alcoholic fatty cirrhosis might be tested better perhaps by a somewhat different type of experiment although it is appreciated that this might be difficult to do in the clinic Choline by itself, or with glucose, cannot be expected to constitute complete therapy In addition to removal of excess fat there must be regeneration of hepatic parenchyma, and the latter requires protein Choline cannot provide this necessary protein It has been suggested only as an adjuvant in the high protein therapy of cirrhosis There should be two groups of patients both receiving an adequate amount of protein or amino acids, but not unusually high in methionine One of the groups should, in addition, receive choline and the possible value of choline should be gauged by the rapidity and extent of the improvement as compared with the noncholine group

In 1944 Doctor Russakoff and I reported a partial approach toward this method Included in a small series of cirrhotic patients with ascites were three who showed no significant improvement after two weeks on a complete diet adequate in protein (protein, 125–200 gm, carbohydrate, 250–300 gm, fat, 50–60 gm) Choline chloride was then added to the regimen in dosages of 3–6 gm per day Marked clinical improvement, with decrease in ascites occurred in all three patients within a week following the start of choline therapy The coincidence of clinical improvement and choline administration suggested that choline might have therapeutic value

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THE PATHOLOGY OF DIETARY LIVER NECROSIS—A PRELIMINARY REPORT

By George L Fite
U S Public Health Service, National Institutes of Health, Bethesda Md

Hepatic necrosis induced by dietary means, although originally observed in [illegible]

[illegible] well established, with deficient protein amino-acid fractions of the diet the common denominator of the several dietary means employed [1, 2, 3, 4, 5]

The necrosis is, in the last analysis, the end result of an underlying degenerative process. Schwarz[6] has introduced the term *dietary necrotic liver degeneration* to emphasize the degeneration as the primary phenomenon Although much information has been gained with regard to substances which will prevent the necrosis from occurring, still the basis nature of the degenerative process behind it is as yet little understood In various reports, necrosis has been observed anywhere from the 30th to the 90th day or later following the placing of animals on a suitable dietary regime, with death of the animals usually following the [illegible]

[illegible] method of Schwarz was the provo[illegible] Torula yeast providing the protein [illegible] This diet has been regarded as being primarily deficient in respect to vitamin E and "Factor 3" From the standpoint of the suitability for histologic study of livers of animals administered this diet, there are certain great advantages, which stem from the consistently high percentage (90–100 per cent) of animals dying with liver necrosis during the span of the 35th to the 50th day, providing many early lesions

In comparing in a general way the lesions of these animals with those described by other writers, using other but not entirely different diets it is possible to make the general statement that the lesions are in every way so similar as to indicate that the problem, the nature of the underlying degeneration is the same Differences are those of degree and time, but not of general character

In addition to changes in the liver in the animals studied, other changes especially in the kidney and in the fore stomach have been seen the latter being of the character known to occur in vitamin E deficiency Although not considered here in detail, they are added evidence of the deficient quality of the diet used

Livers of more than 400 animals drawn from the experiments of Schwarz have been studied, presenting a broad view of the field The problem is complex, and the picture is complex This incomplete report indicates certain lines of investigation of promise for the future and cautions against unwarranted inferences. In general, findings are in harmony with those of other investigators. Interpretations may differ

produced by feeding alcohol or sugar and its prevention by choline Brit Med J 2 1001–1006
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In the current investigations, the "yeast" method of Schwarz was the provocative dietary procedure a special type of Torula yeast providing the protein in a diet complete in most other respects This diet has been regarded as being primarily deficient in respect to vitamin E and "Factor 3" From the standpoint of the suitability for histologic study of livers of animals administered this diet, there are certain great advantages which stem from the consistently high percentage (90–100 per cent) of animals dying with liver necrosis during the span of the 35th to the 50th day, providing many early lesions

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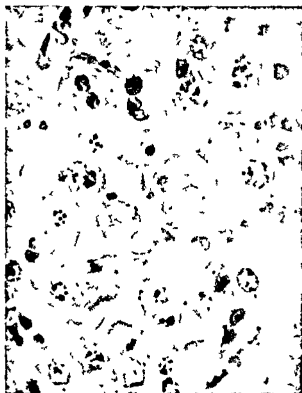


Figure 1 Diffuse karyolysis and karyorrhexis of liver cell nuclei during acute phase

may often appear normal or nearly so In glycogen and fat stained sections it is possible to find many cells containing glycogen but no fat yet illustrating the beginnings of the karyolysis However it is clear that these cells do lose their glycogen in a short time probably a matter of a few hours once the process is started and likewise acquire cytoplasm lipoids at the same time Once the karyolysis is well established the cytoplasm often appears frayed out somewhat watery perhaps increased in over all volume But the point to be made is that the nuclear degeneration often appears to precede the cytoplasmic

It is dangerous to draw too much inference from this histologic change The liver may appear normal on the 33rd day and necrotic on the 34th It is obvious that the histologic evidence of liver cell injury is only a partial measure of what goes on

Another curious finding is the occurrence of numerous mitotic figures in liver cell nuclei in occasional animals which are undergoing this degenerative process The mitoses occur at random anywhere and are far
seen in livers undergoing regeneration Evidently
them as though the primitive mechanism of rep

An experiment was undertaken in which animals were killed at predetermined intervals after being on the basic diet 24, 27, 30, and 34 days a total of 30 animals being used Frank necrosis was seen in two animals killed on the 34th day All livers were sectioned and studied for fat and glycogen

The results paralleled those of Abell, Beveridge, and Fisher,[7] who employed similar procedures The glycogen was normal in all animals except four killed on the 34th day The two which showed early necrosis showed no glycogen In the two others, it was diminished, being found largely in portal areas There were no definite abnormal fat deposits except in the two animals with early necrosis, in which droplets of moderate size were distributed in moderate numbers throughout the liver The progressive increase in fat seen by Abell[7] was not observed in the shorter elapsed time of this experiment

Perhaps the most significant finding of this experiment is the essentially normal histologic appearance of the liver almost up to the point of appearance of the necrosis It seems important to emphasize the fact that although the deficiency will routinely kill animals at about the 45th day, there is little histologic alteration in evidence almost up to the point at which necrosis appears

Types of Liver Cell Degeneration

In the animals dying with or without frank necrosis, two distinctive types of changes in the liver cells had been observed

The first of these is diffuse karyolysis and karyorrhexis of the liver cell nuclei with relatively little change in the cytoplasm of the liver cells (FIGURE 1)

whether, in these animals the deficiency affects primarily the nucleus o cells, or whether one is dealing with a deficiency in substances essential to formation of nuclear protein or essential to nuclear metabolism The strong impression is that the injury to the liver cell in these animals is a very different thing from the injury due to carbon tetrachloride or other chemical substance in which the cytoplasm of the cell must necessarily absorb quantities of the chemical irritant

The sequence of the karyorrhexis-karyolysis process appears to be roughly as follows

(1) A rearrangement of the nuclear chromatin

(2) A diminution in size of the nucleus,

(3) Dissolution of the nuclear capsule,

(4) Contraction of the volume of the nucleus as a whole, yet with outlines preserved

Following dissolution of the nuclear membrane, the nuclear contents do not fuse with the cytoplasm but the nuclear form is retained for a considerable period of time Many nuclei will also shrink to become pyknotic, but this type of degeneration appears to follow later

In cells showing the karyolytic process in its earliest phase, the cytoplasm

killed in both early and subacute stages of the process, as well as in a few animals which had been fed protective substances after the period of potential necrosis was well under way It seems probable that the eosinophilic granulates of the cytoplasm become calcified only infrequently, the determining factors being unknown

Up to the present time, the exact relation of this type of degenerative process to the other is not understood This type of lesion had not been seen too frequently to the point of calcification of the bodies, but it has been possible to demonstrate the early cytoplasmic change in the liver cell many times The mechanism appears to be quite different from that of diffuse karyolysis Here the nucleus is unaltered until after the process of granular body formation is well developed That the granules may develop from altered mitochondria is a strong possibility, proof of which is as yet wanting

The Circulatory Factors

Himsworth and his associates[3, 4] have stressed the circulatory factor in liver necrosis, especially with respect to the distribution of lesions The distribution of the areas of necrosis in these animals has been essentially the same as that observed by all other investigators· more common at the free margins of the lobes, more common where these are in contact with other viscera more common in lobes which are thin, than thick Himsworth went to great lengths to show that a failure of splenic and portal blood to mix promptly might be responsible for differences, in other words, that the differences might be related to a condition when one lobe receives more blood directly from the intestines, while another receives a majority of its venous blood from the spleen While not attempting to repeat all of Himsworth's detailed studies relative to this problem, India ink was injected into some thirty animals on the 33rd day, at a time when liver necrosis was incipient The injections were made in the spleen, and the animals killed a few hours later As nearly as could be judged from gross observation of blackening of the liver immediately following the injection, and from microscopic sections, the ink was evenly distributed to all lobes, and promptly taken up by the Kupffer cells Only minor differences of lobular distribution were observable, such as might be attributed to a temporary increase or decrease of flow to a given area Several animals showed early stages of degeneration, in which the ink was deposited as in normal areas Three animals with early necrosis showed absence of ink from some necrotic areas, but by no means all The only conclusion to be drawn is that any alteration in circulation occurs after and not before, the occurrence of necrosis

A second consideration is the distribution of necrotic areas with respect to the liver lobule Experience has been that there is no absolute rule with respect to the occurrence of areas of degeneration or necrosis, although there are preponderances

(1) There is no focal necrosis Areas of necrosis are invariably larger than the individual lobule

(2) The usual area of frank necrosis involves an area of the liver without respect to its main blood supply Exceptions occur, however, in which the

against destruction has been evoked Although this phenomenon has been seen in only about 2 per cent of all the livers examined, it is too striking to go without mention, and is further evidence of the profundity of the alterations in the liver cell

A *second* type of degenerative change has been observed in many animals, often in those showing varying degrees of diffuse karyolysis, but not conjointly within the same cell This is seen first as fine refractile eosinophilic granules evenly distributed in the cytoplasm These are quite small, of the order of 1–2 μ in diameter When cells containing these bodies become separated from neighboring cells, as they often do, the granules become larger, and finally undergo calcification (FIGURE 2)

The various stages of formation can be followed through various animals, although in a given animal the changes throughout are essentially of the same age and degree The cell form is often retained, perhaps supported by the calcific bodies The nucleus is unaltered at first, later fading out of existence without undergoing karyorrhectic changes

This sequence of events has been seen to best advantage in animals dying or



FIGURE 2 Cytoplasmic granules in degenerating liver cells advanced stage with calcification

Once necrosis has occurred, the resulting effects on the circulation in the

anoxemia may produce still further change In most cases the animal lies [illegible] quickly for this to be histologically clear-cut

A second result of the necrosis is the "hemorrhage" which is seen frequently For the most part this so-called hemorrhage is an accumulation of blo[illegible] sinusoids at the margins of necrotic areas rather than extravasation ou[illegible] the normal channels Another type, however consists of pools ar[illegible] efferent veins suggesting some degree of back tracking of blood from efferent ve[illegible] necrotic areas Again these accumulations are within sinusoid[illegible] hemorrhage (i e the escape of blood from vascular pathways) prol[illegible] not occur

It is impossible to estimate the degree to which simple congest[illegible] operate to provoke frank necrosis Yet when one sees frequently [illegible] degeneration which has occurred in more "normal" areas in the [illegible] livers it is easily supposed that an extremely slight alteration in [illegible] might operate to initiate or to advance the formation of a large area of [illegible] Under the circumstances it hardly seems necessary to conceive a gross alt[illegible] tion or variation in the hepatic circulation to explain the change from degener[illegible] tion to necrosis

Delayed Liver Necrosis

[illegible]

only a certain number of the animals have actually died from necrosis Here the picture may be different in several ways from that of animals dying early in the disease

(1) The picture of diffuse karyolysis is shown to better advantage or to a greater degree in animals dying at about the 50th day than in animals dying on the 35th day on the basic diet This seems to support the idea that the diffuse [illegible]

[illegible] surviving longer periods of time seem to illustrate degenerative processes to better advantage than animals dying in the earlier acute phase

2 Abell[7] speaks of submassive and recurrent submassive necrosis These changes are similar to those we have observed in some animals dying at later time intervals He illustrates the phenomenon of numerous mitotic figures in this "recurrent submassive" variety In our animals histologic evidence of recurrent necrosis has been seen (1) where a delicate balance between death and survival was maintained, or (2) when scars and regenerated areas obviously resulted [illegible]

[illegible] h scars in

[illegible] means at



FIGURE 3 Gross appearance of liver of rat dying with acute necrosis

necrotic areas have an irregular ramifying serpentine pattern analogous to the branchings of *efferent veins*

(3) There is commonly a narrow zone of preserved cells adjacent to portal areas In frankly necrotic areas this is extremely narrow at best In degenerating areas a layer 3 to 5 cells thick around portal zones may be normal

(4) Inversely there are many examples in which the preponderant areas of degeneration or necrosis or both are in a sense centrolobular This is never however a discrete central zone but a broad area making up most of the lobule

It becomes clear that the circulatory arrangement of the liver does have an effect on the distribution of the areas of necrosis in that the more immediately nourished portal cells suffer the degenerative changes slightly less

DIETARY METHODS FOR INDUCTION OF NECROTIC LIVER DEGENERATION

By Marianne Goettsch

University of Puerto Rico School of Medicine, San Juan, Puerto Rico

Necrotic liver degeneration is readily induced in young rats by the dietary control of two factors, sulfur containing amino acids and Vitamin E. I am aware that the modern interpretation requires at least three factors: cystine, vitamin E, and Factor 3, but I shall leave their elucidation to Doctor Klaus Schwarz. Doctor Daft has given an interesting account of the historical sequence of factors in nutritional liver injuries. The preventive effect of vitamin E [illegible]

[illegible]

recognized the importance, in experiments of this type, of restricting [illegible]

[illegible]

1951), which was the protein used by the original investigators. The composition of the diets is given in TABLE 1. Diet 1 is typical of diets used in Vitamin E experiments. Diet 2 contains the same ingredients as the low protein diets 3 to 6. When α tocopherol was added, it met the minimum protein requirement for growth and reproduction in several generations of rats.

Supplements were introduced at the following concentrations: DL methionine, 0.1 to 0.3 per cent; L-cystine, 0.1 to 0.3 per cent; and dl-α tocopherol acetate, 2 mgm. per rat per week.

Procedure. Wistar or Sprague Dawley rats were used. Breeding rats were reared and bred on the low vitamin E but adequate diets 1 and 2, which were equally suitable for the preparation of young. To permit the birth of young the rats were given 10 mgm. of α tocopherol at the beginning of the first gestation, 20 mgm. at the second, and 30 mgm. at the third. The young did not develop muscular dystrophy at the end of lactation. In this way, young with initial low stores of vitamin E were obtained.

These young rats were given diets 3 to 6 from the 21st day, at which time the body weight was approximately 35 gm.

Results and Discussion

Rats with the low casein diets grew poorly. Nearly all of them, about 90 per cent, died suddenly after 5–40 days. In recent trials the incidence reached 100 per cent and none of the rats survived for longer than 20 days. Liver necrotic degeneration was observed grossly and confirmed by Doctors Koppisch and Izquierdo of the Department of Pathology of the University of Puerto Rico School of Medicine by microscopic examination. Some of the rats presented early lesions of muscular dystrophy.

any time prior to its actual occurrence In a number of animals so protected, and killed later, the appearance of the liver has usually been that of the normal animal In a few, scarring and regeneration have appeared Except for these evidences of repair and regeneration of frankly necrotic areas, no changes have been seen Thus present indications are that the degenerative changes are reversible to a substantial degree although the limits have yet to be defined

Cirrhosis has never been observed in these animals on the necrogenic diet nor has the picture of the fatty liver of choline deficiency been seen

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Discussion of the Paper

DOCTOR HANS POPPER, *Hektoen Institute, Chicago, Ill* I should like to congratulate Doctor Fite on a thorough and much needed investigation upon the histologic changes in acute hepatic necrosis It is worth emphasizing that histologically insignificant changes precede the explosive sudden breakdown of the liver cells and that nuclear changes usher in the lesion The eosinophilic necrosis described is also interesting from the point of view of the human

[illegible]

tions granular coagulation necrosis of the cytoplasm of the character of Mallory bodies *is more common* This raises the question as to the general behavior of the cytoplasmic basophilia supposedly due to the presence of pentose nucleic acid in nutritional necrosis I also should like to ask about possible alterations of the reticulum framework in the animals studied In most instances of human hepatic necrosis presumably produced by either viruses or chemical toxins *it remains intact whereas it is broken in some types of toxic hepatitis* and as the result of ischemia Obviously, the reticulum changes would be of great importance in recovery stages from such necrosis

low E—high casein diet until they weighed 70–100 gm With increasing initial body weight, there was a decrease in the incidence of liver necrotic degeneration and an increase in the time of survival This observation confirms that of Lindan and Himsworth (1950)

Initial stores of vitamin E in the rat There is great variation among rats in their ability to store vitamin E in the tissues and in the requirement of vitamin E for reproduction and for the prevention of muscular dystrophy In vitamin E low rats that were given one single dose of 2 mgm α tocopherol on the 15th day of lactation, the incidence of liver necrotic degeneration was reduced 50 per cent

The discordant results among different laboratories in the induction of liver degeneration presumably have been associated with the amount of α tocopherol supplied during the pre experimental period It was recently observed that some rats reared and maintained with a commercial ration, underwent resorption gestations when they were given the low E—high protein diet from the beginning of gestation These results suggest an explanation for the induction of liver necrotic degeneration in rats that have not been purposefully prepared to contain low initial stores of vitamin E

Addition of methionine and cystine The preventive effect (Weichselbaum 1935 Daft Sebrell, and Lillie, 1942) of the sulfur containing amino acids is well known Although these experiments were not quantitative as were those of Schwarz (1952), the addition of 0 3 per cent of either DL-methionine or L-cystine to the low casein diets prevented the liver necrosis in a high proportion of the rats under the given conditions As with other groups of rats chronic muscular dystrophy and sterility developed in all rats that did not

... of α tocopherol Liver necrotic degeneration was prevented in all rats receiving 2 mgm α tocopherol per week, in confirmation of the results of Schwarz (1944) Gyorgy (1947) and Gyorgy and Goldblatt (1949) The α tocopherol not only prevented liver necrotic degeneration but also protected the rats from muscular dystrophy and sterility When the rats were sacrificed after a st...

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injury was highest among rats with the high lard diets The addition of 0 3 per cent ...

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Summary

(1) With low E—low-crude casein diets liver necrotic degeneration appears rapidly in young rats deprived of Vitamin E Early lesions of muscular dystrophy may occur

TABLE 1

COMPOSITION OF EXPERIMENTAL VITAMIN E-LOW DIETS

Constituents*	Diets for breeding rats†		Isocaloric low protein diets			
	1	2	3	4	5	6
Crude casein	26 6	20 0	7 5	8 3	10 0	11 4
Yeast brewers type	16 6	—	—	—	—	—
Cornstarch	33 3	68 0	86 5	79 7	64 0	48 0
Salt mixture‡	3 4	4 0	4 0	4 0	4 0	4 6
Lard	18 3	6 0	—	6 0	20 0	34 0
Cod liver oil	1 8	2 0	2 0	2 0	2 0	2 0

After some weeks, the survivors ceased to grow and acquired the chronic muscular dystrophy that was described by Einarson and Ringsted (1938) in low E rats A few that survived 90 days were bred. They manifested the typical sterility of vitamin E deprivation (Evans and Bishop, 1923, Mason 1926) At death, these rats presented normal liver The reason for this result is unknown *It may be that the wasting away of muscle releases* sufficient of the sulfur containing amino acids to protect the liver

Influence of lard and cod liver oil Lard and cod liver oil are important variables in the induction of vitamin E sterility in rats

The incidence of liver necrotic degeneration was not influenced by variations from 0 to 34 per cent in the lard content of the isocaloric diets The results were not altered with three different types of lard modern processed old type or Eastman low E In other diets, afaxin and viosterol were substituted for the cod liver oil All of the rats developed fatal hepatic necrosis It appears that the disease occurs in the absence of cod liver oil and is independent of the lard concentration of the diets, under the given conditions

Variations in the content of casein The protective effects of high casein diets are well known The crude casein concentration was varied from 5 8 to 14 0 per cent in diets containing 6 per cent lard All of the rats with the 5 8 per cent diet 2 of 6 with 11 6 per cent casein and none of 24 with 14 0 per cent casein acquired liver necrotic degeneration Chronic muscular dystrophy appeared in all rats that did not develop liver lesions They survived for 4 to 6 months, presenting severe muscular dystrophy and normal liver at death

Other sources of protein Under the given conditions liver necrotic degeneration has been observed with low E—low protein diets (Goettsch, 1948 1951)

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ally grown),

ins contain

the factor 3 of Schwarz (1951) is not known

Initial size of the low-E rat Since it has been customary to use rats with an initial body weight of 70–100 gm , young low E rats were maintained with the

DIETARY HEPATIC NECROSIS IN THE RAT—ABSENCE OF CIRRHOSIS FOLLOWING RECURRENT EPISODES*

By F W Hoffbauer and Bernadine Wittenburg
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The investigations to be described arose from an interest in the human disease postnecrotic cirrhosis, and represent an attempt to produce an experimental form of the disease This type of cirrhosis presents a challenge to the pathologist and to the clinician The etiologic factor or factors responsible are obscure The morbid anatomy is variable The clinical course is, however, fairly constant. Patients with postnecrotic cirrhosis quite uniformly exhibit a gradual but progressive deterioration of hepatic function and the disease ultimately proves fatal The clinical course and the rate of progression of the disease is subject to great individual variation We are not convinced that [illegible]

[illegible] rhosis do follow an attack of viral hepatitis

There is a similarity between one form of cirrhosis seen in the clinic (the patient who is a victim of chronic alcoholism and consequent malnutrition) and one form of experimental cirrhosis (the rat that is a victim of prolonged fatty infiltration of the liver) The investigator possesses a laboratory method by which he can produce a disorder that resembles in many respects a disease that he encounters in the hospital wards In attempting the production of an experimental form of postnecrotic cirrhosis, an obvious approach would be to cause chronic viral hepatitis in an animal This most desirable accomplishment seems quite unobtainable at the present time Hence, we sought to produce a structural counterpart of this disorder by inducing liver damage through a mechanism that was available The lack of any etiologic similarity between the method used, experimental dietary necrosis, and viral hepatitis was recognized and appreciated.

In the experimental production of any type of cirrhosis, repeated exposure to the insulting or damaging agent is an essential feature When carbon tetrachloride is employed for this purpose, the investigator administers the toxic agent twice a week, three times a week, week after week, until he obtains the desired goal In the case of fatty infiltration, the damaging effects of the fatty cysts proceed continuously night and day in the fashion that Hartroft[1] has demonstrated Would it be possible to produce a structural counterpart of human postnecrotic cirrhosis if an animal could be forced to undergo repeated episodes of dietary liver necrosis? The following studies were directed toward such a goal There are two aspects to be reported (a) the resultant pathologic changes observed after the spontaneous occurrence of repeated attacks of dietary necrosis, (b) the resultant changes noted when one attempts to modify the course of events after one or more episodes of necrosis have occurred

* These investigations were sponsored by the Commission on Liver Disease Armed Forces Epidemiological Board and supported in part by a contract from the Office of the Surgeon General U S Army

(2) These lesions develop in the absence of cod liver oil and are independent of the lard content of isocaloric diets

(3) They occur

ers' type and torul

(4) An increase

or methionine, decreases the incidence of liver degeneration and permits the

tion enabling the rat to survive for a long period of time and to reproduce under conditions that are associated with fatty deposition in the liver and cirrhosis Methionine prevents, and cystine intensifies, fatty infiltration of the liver and diffuse hepatic fibrosis

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Sch er degeneration in

Sch rotic liver

Tuc onine and

Wei J Physiol 25 363–367

TABLE 2

INCIDENCE OF POSTNECROTIC SCARRING OF THE LIVER IN RATS DYING OF DIETARY NECROSIS

No. of rats	Sex	Initial weight (± 10%)	Diet*	Survival period (days)	Degree of Scarring		
					Absent	Minimal	Marked
10	M	50 gm	H2	34–72	5	4	1
18	F	50 gm	H2	27–74	11	4	3
10	M	100 gm	BY18	63–116	3	7	—
10	F	100 gm	BY18	64–144	6	4	—
9	M	100 gm	TY20	43–137	8	1	—
13	F	100 gm	TY20	74–202	8	5	—
3	M	100 gm	TY35	72–106	—	3	—
5	F	100 gm	TY35	118–180	3	2	—
17	M	50 gm	BY18 R	25–59	2	10	5
0	M	50 gm	BY18	28–70	21	32	17

*Diets H2—18% Baker's yeast corn starch and oil (see Text) BY18—18% Baker's yeast sucrose and lard (see Text) TY20—20% Torula yeast sucrose and lard (see Text) TY35—35% Torula yeast sucrose and lard (see Text) BY18 R—1 ml. resin (Resion) added to daily ration (see Text)

completion of an experiment, all specimens were graded as to the degree of scarring Appropriate sections were then removed for histologic study A massive hemorrhagic necrosis of the liver was the cause of death in all rats except as described subsequently (TABLES 4 and 5) This lesion can be detected by gross inspection The presence of scarring indicative of one or more previous episodes of submassive necrosis can also be detected by careful gross inspection The scarring was graded as *minimal* if the left lateral lobe alone was involved and as *marked* if one or more additional lobes revealed the characteristic pits or furrows Microscopic sections were prepared by standard procedures. Hematoxylin eosin and azocarmine stains were used most frequently In a few instances, plastic corrosion preparations of the intrahepatic veins were made by injecting colored vinyl acetate solutions into the portal and hepatic veins

RESULTS

The presence of depressed scars in the liver of a rat dying of dietary liver necrosis is an indication that the animal has undergone one or more previous episodes of liver cell degeneration The sequence of events that leads to the development of such lesions has been well described by Himsworth[4] and by Abell, Beveridge, and Fisher[5]

The …

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exp …

was … The addition of the resin compound had no influence … course of the disease or the resultant pathology (the 17 controls f… periment are included among 70 males in TABLE 2)

TABLE 1
COMPOSITION OF DIET BY18

Sucrose	72%
Yeast[1]	18%
Salts[2]	4%
[illegible]	1%
[illegible]	0 05%
[illegible]	4 95%

[1] Dried B k ng Yeast [illegible]

[illegible]

ca [illegible]
of [illegible]

anc [illegible]
of ration

[5] A special lard tocopherol content minimal †

* Kindly made available by Ayerst, McKenna and Harrison Ltd New York 16 New York
† Kindly made available by Hormel Company Austin Minnesota

Experimental Methods Employed

Throughout these experiments, a uniform strain of white laboratory rats* was used Male rats weighing 50 ± 5 gm at the start of the experiment were routinely employed except in the initial investigations (described in TABLE 2) The animals were housed in individual cages suspended in racks of a type that provided a continuous sheet of semi waterproof paper beneath the cages This arrangement proved convenient, since daily urine tests for bilirubin were necessary Specimens for testing were secured from small pools of urine that collected beneath each cage Franklin's[2] method for detecting bilirubin in the urine was employed routinely in these studies Daily urine tests were begun 20 days after the animals were started on the necrogenic diet.

Diet The diet employed in the major portion of this study was designated by BY18 The composition is given in TABLE 1 Each rat was allowed 8 gm. per day Fresh rations were mixed once each week and refrigerated The animals were fed three times each week Food consumption was not routinely measured Free access to water was allowed

In preliminary investigations diets of slightly different composition were given as indicated i [illegible] ribed in a previous report [3] [illegible] id [illegible] in 35 percent *Torula* [illegible] only two respects There was a reduction in the sucrose content to 70 and 55 per cent respectively The vitamin mixture employed did not contain vitamin K The diet BY18R differed only in that 1 ml of a liquid resin preparation‡ was added to the 8 gm ration allowance offered daily to each animal

Pathological analysis Animals were autopsied as soon after death as possible Where the animals were obviously moribund they were anesthetized and sacrificed The livers were fixed in a neutral formalin solution At the

* Purchased from Holtzman Rat Company Madison 5 Wisc
† Supplied by the Sulphite Pulp Manufacturers Research League Appleton Wisc
‡ RESIONR supplied by the National Drug Co Philadelphia Pa

Table 1
Experiment 1
Incidence of Deaths in Rats Given Experimental Diet*

Group	Management: Housing · Usual range of temperature °F	Management: Feeding	No. tested	No. dead with acute liver necrosis	Median survival time (days)	No. dead without acute liver necrosis
A	Animal house (63–70°) (heated)	"Ad lib"	8	7	19	0
B	Animal house (63–70°) (heated)	Restricted	9	1†	—	0
D	Rabbit hutch (40–50°) (unheated)	"Ad lib"	8	0	—	0
E	Rabbit hutch (40–50°) (unheated)	Restricted	9	0	—	4

* Survivors killed 53 days after feeding low-protein diet
† Rat died 53 days after feeding low-protein diet

died of acute liver necrosis while those with a restricted food allowance or those in the lower environmental temperature (40–50°F) did not develop the disease. When the food allowance was restricted in the lower environmental temperature four among nine rats died without liver necrosis.

The second and third experiments were conducted in the winter of 1949–1950 and in the spring of 1950 with better temperature control of the heated environments. In experiment 2 the usual range of the air temperature of the unheated environment was 40–50°F, that of the heated environment 70–74°F, i.e., several degrees Fahrenheit above the temperature studied in experiment 1. In experiment 3, groups of rats in two heated environments 70–74°F and 60–65°F, and two unheated environments were studied. In this experiment, only one 'restricted' group of rats was studied (group 3E TABLE 2). These rats were put into the environment of 60–65°F, so that the conditions at the beginning of experiment 1 we

firmed those of exp

respects (1) in the

liver necrosis was the same among the rats "restricted" in their food allowance

Table 2
Experiments 2 and 3
Incidence of Deaths in Rats Given Experimental Diet

Experiment	Group	Management: Housing · Usual range of temperature °F	Management: Feeding	No. of rats tested	No. dead with acute liver necrosis	Median survival time (days)	No. dead without liver necrosis
2	A	Animal house (70–74)	"Ad lib"	9	8	43	0
	B	Animal house (70–74)	Restricted	9	8	40	0
	F	Unheated wooden shed (40–50)	Ad lib	10	3	—	0
	F	Unheated wooden shed (40–50)	Restricted	10	1	—	3
3	A	Animal house (70–74)	Ad lib	12	11	19	0
	B	Heated wooden shed (60–65)	Ad lib	12	8	26	0
	D	Rabbit hutch (35–45)	Ad lib	12	0	—	1†
	E	Heated wooden shed (60–65)	Restricted	8	0	—	0

† Killed because of severe otitis media on 21st day after being first given the experimental diet

THE EFFECT OF ENVIRONMENT AND MODE OF FEEDING AND OF REARING ON THE PRODUCTION OF ACUTE DIETARY LIVER NECROSIS IN THE RAT

By J M Naftalın
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My aim in the experiments described below was to define the conditions under which young male rats would become susceptible to acute dietary liver necrosis

In the first series of five experiments,[1, 2] two conditions were examined simultaneously, (1) the influence of environment, and (2) the mode of feeding *i e* whether the rats were allowed to eat to appetite or not In the second series of three experiments[3] a method was found of adjusting the susceptibility of groups of rats to the development of acute liver necrosis With such a method more detailed comparisons can be made between rats likely to develop liver necrosis in a short time and those which would take longer to do so or would not develop the disease at all

The Interactions between Environmental Temperature and Mode of Feeding

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Diets The following three diets were used (1) During pregnancy and the first 17 or 19 days of lactation, the Rowett Research Institute stock rat cube together with cow's milk was given This diet was followed by (2) a semi synthetic preliminary high protein diet containing casein 16 or 19 (vitamin free ' Glaxo Laboratories Ltd, Middlesex, England), brewers' yeast 3 (Phar Chemical Products, Ltd, London), sucrose 68 or 65 commercial lard 7 ... 4, cod liver oil (B P) 2 per cent, and synthetic B vitamins but ... Vitamin E These diets were available to the young (3) week of age, the young were fed a low protein diet containing of protein, 8 per cent casein and 3 per cent yeast the sucrose ...ed to 76 per cent The yeast was added to supply the extra ... red by animals exposed to cold The reasons for ... lained in a previous paper[1]

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* Surv vors k lled 53 days after feed ng low protein d et
† Rat d ed 51 days after feeding low-protein d et

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TABLE 2
EXPERIMENTS 2 AND 3
INCIDENCE OF DEATHS IN RATS GIVEN EXPERIMENTAL DIET

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[illegible] of experiment 1 were simulated. The results (TABLE 2) of experiment 3 confirmed those of experiment 1, while those of experiment 2 differed in two main respects: (1) in the heated environment of 70–74°F, the incidence of acute liver necrosis was the same among the rats "restricted" in their food allowance

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Results

The results of the first experiment are shown in TABLE 1 It is evident that in an environmental temperature of 63–70°F the rats which ate to appetite

TABLE 4

RESULTS OF FOOD RESTRICTION AT VARIOUS ENVIRONMENTAL TEMPERATURES
(*Experiments* 1–5)

Environmental temperature F (usual range)	Expt no	Group average degree of food restr ct on	Results (restricted groups)
88–92	5	1 7	Death without liver necrosis
		1 2	Protection against liver necrosis Rats live
"0–78	4	1 6	Not complete protection against liver necrosis
		1 3	No protection against liver necrosis
0–74	2	1 2→1 1	No protection against liver necrosis
60–64	4	1 6	Death *without* l ver necrosis
	3	1 6→1 4 (lowest 1 25)	Protection against liver necrosis Rats live
	4	1 2	No protection against liver necrosis
About 63	1	1 15, 1 09*	Protection against liver necrosis.
Below about 45	1 & 2	When *ratio* was about 1.35	*Death without liver necrosis.*

* Only 2 rats stud ed for one week in the corresponding "Ad Lib" group

at the higher environmental temperature of 70–78°F, the rats which were

degree of food restriction was 1 2 in the environmental temperature of 60–64°F The degree of food restriction necessary to prevent death at 60–64°F would seem to lie about 1 4—the ratio used in experiment 3 (TABLE 4)

Experiment 5 was an extension of these observations Littermates were

ment From this experiment it was concluded that an environmental temperature of 88–92°F was higher than the optimum temperature for the produ ction of acute liver necrosis[3] This conclusion was reached because, at this temperature the group of rats allowed to eat to appetite survived longer than their littermates housed at 74–76°F, and because the low degree of food restriction of 1 2 prevented liver necrosis. The range 80–88°F has not yet been stu l ed but an environmental temperature of about 75°F is suitable for a h h inci lence of the disease It was thus apparent that *it was the degree of food restriction in relation to the environmental temperature that determ ned whether a rat survived or d ed either of liver necrosis or of inanition* (TABLE 4) Although the tren l is clear it should not be inferred that these ratios are fixed

TABLE 3

EXPERIMENTS 1, 2, AND 3

FOOD EATEN BY "AD LIB" GROUPS* AND DEGREE OF FOOD RESTRICTION

Expt no	Envirn temp °F Usual range (extreme range)	Average food eaten g /rat/day by 'ad lib group ( )	Average food offered g /rat/day to restricted group ( )	Average degree of food restriction ad lib / restricted	Remarks
1	63–70	5 8 (A)	5 0 (B)	1 15, 1 09	i. Only 2 rats studied for 1 week in ad lib" group A ii One rat out of 9 died with liver necrosis in restricted group B when environ mental temp reached 80°F
	40–50	11 8 (D)	8 8 (E)	1 3	Four rats died *without* liver necrosis in restricted group E Degree of restriction greater than 1 3 at that time
2	70–74	4-5→3 9 (A)	3 6 (B)	1 2→1 1	Incidence of liver necrosis the same in groups A and B, *i e*, 8 out of 9
	40–50	10 8 (E)	7→9 4 (F)	1 2	Three rats died *without* liver necrosis in group F when degree of restriction was 1 4, 1 34 and 1 35 Temps 38, 28, and 40°F
3	60–65	7 0 (B)	4→6 (E)	1 6→1 4	No rat died in restricted group E

* Only groups relevant to the argument are shown For details of other groups see Naftalin
→ = increased or reduced as experiment progressed

(group 2B TABLE 2) as among those fed "ad lib" (group 2A), (2) the median survival times, after first giving the experimental low protein diet, were 40 and 43 days, as compared with 17 and 19 days in experiments 1 and 3 The reason for this second difference is not fully apparent, though it may be due to seasonal changes

In seeking to explain why the "restricted" rats in experiment 2 behaved differently from the "restricted" rats in experiments 1 and 3, the amounts of food eaten and the degree of food restriction were then examined The results are shown in TABLE 3 In experiment 2, the rats fed "ad lib" (group 2A) did not eat as much as those in corresponding groups in experiments 1 or 3 (groups 1A and 3B) The average amount eaten by group 2A decreased from *4 5 to* 3 9 gm /rat/day as the experiment progressed It had been decided at the beginning of experiment 2 to offer 3 8 gm of food daily to each rat in group 2B the "restricted" group but, although after 8 days this was reduced to 3 6 gm, the ratio of food eaten by the group fed "ad lib" to the "restricted" group did not rise above 1 1 It seemed, therefore, that the degree of food restriction had not been great enough at 70–74°F to prevent death from acute liver necrosis This postulate was tested in experiment 4, when two degrees of food restriction were studied at two environmental temperatures, 60–64°F and 70–78°F The degrees of food restriction were such that the amounts of food

EFFECT OF DIFFERENT MAKES OF CASEIN ON THE PRODUCTION OF ACUTE DIETARY LIVER NECROSIS IN THE RAT

By J M Naftalin
Rowett Research Institute, Bucksburn, Aberdeenshire, Scotland

From 1949 till the summer of 1951, the casein used in diets to induce acute liver necrosis in rats was the "vitamin free" product of Glaxo Laboratories, Ltd., Middlesex, England When this casein became unavailable commercially, "low vitamin" content casein of Genatosan, Ltd , Loughborough, England, was substituted. The next two experiments were conducted in the autumn of 1951 under those conditions which had been found to be most favorable for the production of the disease, *i e*, when the rats were housed in an environmental temperature of about 75°F and allowed to eat to appetite In these latter experiments,[1] no rat suffered from liver necrosis This paper reports in more detail the results obtained by a direct comparison, using littermates, of two batches of casein, Genatosan casein "low vitamin" content, batch no 078689, supplied in 1951, and Glaxo lactic casein "C" (unextracted), batch no UA529, supplied in 1947 It was not possible to make a direct comparison with the sample of Glaxo "vitamin free" casein used in previous experiments.

Methods

Two experiments lasting from February to April, 1952 and from April to July, 1952 are described The environmental temperature of the animal house was 74°–78°F Young male rats of the hooded Lister strain, Rowett Institute stock were weaned on their 17th day instead of their 24th day, as is customary in this Institute, as it had been found that groups with a higher degree of sensitivity to liver necrosis were thus obtained[1, 2]

The *diets* contained casein 16 (Glaxo lactic casein "C" unextracted or Genatosan casein low vitamin content) brewers yeast 3 (Pharmaco-Chemical Products Ltd London) sucrose 68 commercial lard 7 McCollum's salts 4 cod liver oil (B P) 2 per cent choline chloride 200 mgm /100 gm diet, and B vitamins but without added vitamin E These diets were offered from the 17th to 3 th day of age and were followed on the 38th day of age by diets in which the casein was reduced to 8 per cent and the sucrose increased to 76 per cent The rats were always allowed to eat to appetite

[illegible]

a week from their 38th day of age Group C was given diets containing Glaxo lactic casein C In experiment 2 six male rats from each of twelve litters were allocated at random to 2 groups D and E Three rats from each litter were put into each group and fed on diets containing either Genatosan casein or Glaxo lactic casein C

The reasons for the differences between the rats weaned early or late are under investigation. *Since the preliminary high protein diet fed to the mothers* from the 17th day of lactation may in itself lead to necrosis in the young,[3] it would seem that the rats weaned later either (1) had a nonspecific improvement in their nutrition or (2) they received *more of a protective substance* which, (a) although present only in traces in the diet, could be concentrated in the milk or which (b) was synthesized in the mother's body and secreted in the milk.

Summary

(1) A high incidence of acute liver necrosis was produced in young male

cent lard, 2 per cent cod liver oil (B P), 76 per cent sucrose, and 4 per cent McCollum's salt and B vitamins, but without added vitamin E.

(2) Of the ranges of environmental temperature studied, the best temperature for the production of acute liver necrosis was *70°–78°F*.

(3) At all environmental temperatures studied, the incidence of acute liver necrosis was reduced or prevented by a sufficient degree of food restriction, but *too severe a degree of food restriction led to death without liver necrosis*. Whether a rat survived or died either of necrosis or of inanition depended on the degree of food restriction in relation to the environmental temperature and *not simply on the total amount of food eaten*.

(4) Groups of rats weaned on their 17th day of age suffered a higher incidence of acute liver necrosis and had a shorter survival time than their littermates weaned on their 25th day.

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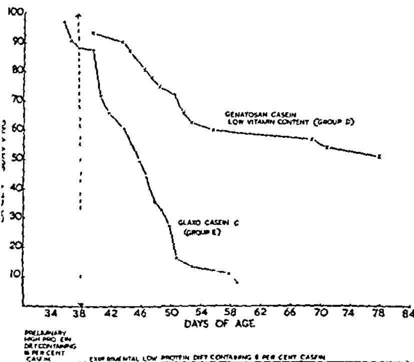


FIGURE 1. Graph of experiment 2. Percentage of rats surviving when fed on diets containing either Genatosan casein "low vitamin" content or Glaxo casein "C". In group D (Genatosan casein) one rat died on 53rd day of age from lung hemorrhage, one rat died on 69th day of age from severe diarrhea and one rat was killed on 80th day of age. None of these rats had liver necrosis and are omitted from the calculation of percentage surviving. In group E (Glaxo casein "C") four rats died from liver necrosis before the low protein diet was fed.

origin.[8] Genatosan casein "low vitamin" content prepared by acid precipitation followed by extraction with methylated spirits and ether, is usually made from British liquid milk, although reconstituted dried skim milk of New Zealand origin is sometimes added if a sufficient quantity of liquid milk is not available.[7] In two earlier experiments[2] when no liver necrosis resulted, an unnumbered batch of Genatosan casein "low vitamin" content was used. In the experiments 1 and 2 of the present paper batch No. 078689 of Genatosan casein "low vitamin" content was made from a mixture of milks of British and New Zealand origin. Unfortunately no information is now available about the unnumbered batch of the earlier experiments. The differences between caseins prepared from British or New Zealand milk may be due to age or to differences arising in preparation or to differences in the milks themselves, which may arise from differences in the feeding systems.

That caseins differ in their ability to lead to liver necrosis was first reported by Schwarz in 1944.[4] Despite this, many workers have omitted to mention the brand of casein they employ. Schwarz's casein VI which induced liver necrosis may have been regarded as specially treated casein because he prepared

TABLE 1

INCIDENCE OF ACUTE LIVER NECROSIS IN EXPERIMENTS 1 AND 2

Expt no.	Group	Make of casein use in diets	No of rats tested	No dead with acute liver necrosis	Median survival time days‡	Remarks
1	A	Genatosan* casein "low vitamin" content	2 from each of 5 litters = 10	4	—	One rat died on each of the following days after feeding experimental diet, 11, 12, 24, 28
	B	Genatosan casein "low vitamin" content + vit B_{12}**	2 from each of 5 litters = 10	1	—	One rat died 8 days after feeding experimental diet
	C	Glaxo† lactic casein "C" unextracted	2 from each of 5 litters = 10	9	7	Two rats died on each of the following days after feeding experimental diet, 4 5 7 and three on the 13th day
2	D	Genatosan casein "low vitamin" content	3 from each of 12 litters = 36	16	—	See graph
	E	Glaxo lactic casein "C" unextracted	3 from each of 12 litters = 36	33	9	See graph

* Genatosan Ltd Loughborough, Leicestershire England.
** Vitamin B_{12} Cytamen (Glaxo) 0 5 μg in 0 1 ml. water given by mouth 3 times a week from 38th day
† Glaxo Laboratories Ltd Greenford Middlesex England
‡ Days after feeding experimental diet i e days after 38th day of age Survivors killed on 47th day in experiment 1 and on 72nd day in experiment 2

Results and Discussion

The

this vitamin was given to one group of ten rats (group B - TABLE 1) The addition of this vitamin may have delayed necrosis, but the numbers studied were too small for the result to be statistically significant

In experiment 2, among the rats fed on the diet containing Glaxo lactic casein "C" (group E), 2 died from acute liver necrosis on their 36th day of age, another on its 37th

per cent of his casein VI obtained a high incidence of liver necrosis In an earlier experiment,[3] in which a diet containing 19 per cent Glaxo "vitamin free" casein was used, one rat among five died from acute liver necrosis The results of experiment 2 are further evidence that a high level of protein *per se* in the diet does not necessarily prevent dietary liver necrosis.

Glaxo lactic casein "C" batch UA529 was a crude product of New Zealand

liver necrosis As a result of their studies with a water washed casein and an oxidized casein, Hove, Copeland, and Salmon[8] came to the conclusion that acute liver necrosis or massive lung hemorrhage in rats depended on a simul taneous deficiency of Vitamin E and a casein co factor More recently Schwarz[9, 10] has reported that a substance ("Factor 3") which prevents liver necrosis is present in a variety of natural materials including American crude casein and "vitamin free" casein prepared by repeated alcohol extraction. It should be emphasized that Glaxo lactic casein "C" (unextracted) is a crude product and therefore, it is not possible to state without further investigation whether the difference between its liver necrogenic properties and those of Genatosan casein 'low-vitamin" content is due to a difference in content of "Factor 3" or whether there is some other reason

Summary

(1) There was a higher incidence of acute liver necrosis amongst rats housed in an environmental temperature of 74–78°F and allowed to eat to appetite when the casein in the diet was the lactic casein "C" (unextracted) of Glaxo Laboratories Ltd, Middlesex, England, than when the 'low vitamin" con tent casein of Genatosan Ltd, Loughborough England, was used

(2) Glaxo lactic casein 'C' (unextracted) is a crude product of New Zealand origin Genatosan casein "low vitamin' content is prepared from British liquid milk with or without the addition of reconstituted dried skim milk of New Zealand origin

Acknowledgments

I am indebted to Dr W F J Cuthbertson of Glaxo Laboratories Ltd, a
to Mr Todd of Genatosan Ltd, for information about the prepa
of used in the experiments described in this paper

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THE INFLUENCE OF THE ENDOCRINE GLANDS ON THE DEVELOPMENT OF ACUTE MASSIVE LIVER NECROSIS

By J. M. R. Beveridge
Queen's University, Kingston, Ontario

The first indication that the endocrine glands probably had some influence on the development of dietary liver necrosis was given in 1935, by Weichselbaum[1] in his paper on the nutritional requirement of the rat for methionine and cystine. The lethal liver lesion that was produced in his rats on a diet low in these amino acids was almost undoubtedly acute massive hepatic necrosis, although no histological description of the tissue was given by him. In this publication, he refers to the fact that the mortality was definitely less in the females. Presumably, this difference is due directly or indirectly to the action of the sex glands. Similar observations have been made by György[2] and by our group.[3] Our results, utilizing rats of average initial weight 129 gm. and in an experiment terminated after 105 days, are shown in TABLE 1. Although the mortality is essentially the same, the average time at which death occurred is significantly longer in the females.

An interesting observation has been made by Ferret[4] to the effect that diets designed to induce liver necrosis caused continuous oestrus in ovariectomized animals in which 5 mgm. estradiol had been implanted into the spleen. The addition of cystine to the diet restored the ability to inactivate the estrogen. On the other hand, alpha tocopherol did not have a similar action, and it must be concluded, on the basis of this work at least, that the inability of animals to inactivate estrogen has a negligible significance in the pathogenesis of liver necrosis.

During an investigation of hepatic damage caused by the feeding of alkali treated casein to rats, Schwarz[5] in 1944 noted that an extract of lipids from the adrenals inhibited the development of the lesion.

In 1951, the same worker[6] reported the results of further studies on the role of the adrenals in the development of the type of dietary massive liver necrosis currently being discussed. Proceeding on the assumption that lack of glycogenesis is one of the essential features of liver degeneration and recalling the role of adrenal cortical secretion in promoting this process, he tested the effect of cortisone on rats being fed a basal necrogenic diet low in methionine, cystine and alpha tocopherol.

The survival time was significantly and equally prolonged in both males and females, but there was no difference in the mortality due to massive liver damage (TABLE 2). This investigation showed clearly that cortisone ameliorated the necrogenic effect of the basal diet.

However, the assumption that imperfect liver glycogenesis is initially involved in dietary liver necrosis is not supported by some of our previous work. One of the first investigations carried out by us[7] on entering into this field was a study of progressive structural and biochemical changes occurring in the liver prior to and at the time of the acute necrotic episode. No change oc

TABLE 1
EFFECT OF INITIAL WEIGHT AND OF SEX ON THE DEVELOPMENT OF LIVER NECROSIS

	Sex	Rats that d ed of necros s or were killed when mor bund		Rats k lled at 105 days		Total incidence of nec osi	
		Rats	Days on diet (av range)	No showing s gns of necros s	No showing no signs of necros s	No of rats	Per cent
Y	M	16	58 (35–97)	3	1	19/20	95
YF	F	16	79 (48–103)	—	4	16/20	80

TABLE 2
RESPONSE OF MALES AND FEMALES TO CORTISONE (NO OF ANIMALS IN PARENTHESES

	Average surv val time (in days)	
	Males	Females
Without cortisone	41 5 (6)	50 1 (4)
With cortisone	57 0 (5)	65 2 (4)
Difference due to cortisone	15 5 days	15 1 days

From Schwarz [9]

curred in liver glycogen levels until at the time of the acute episode when as one would expect, the glycogen levels dropped essentially to zero Apparently liver glycogenesis proceeds unimpaired until signs of hepatic damage appear

Although the nature of the effect of the thyroid hormone on the development of liver damage due to a variety of causes in both experimental animals and in humans is controversial, there is complete agreement on the nature of the effect of the thyroid secretion on the development of acute massive liver necrosis seen in rats fed diets slow in alpha tocopherol and in the sulfur containing amino-acids Handler and Follis[8] first demonstrated the potentiating effect of desiccated thyroid when it was added to a basal necrogenic diet

The diet used by these investigators contained 0 2 per cent desiccated thyroid and caused acute liver damage in about 50 per cent of 50 gm male rats at an average interval of 40 days compared to an incidence of 100 per cent in 70 days for similar rats on the basal diet The diet (TABLE 3) used by us[10] contained

... 99 per cent necrosis after
the basal diet
yroid tissue on
lthiouracil on

the other hand, is not so clear cut With a level of 0 3 per cent p opylthiouracil the rats died at an average interval of 47 days but they showed no evidence of hepatic injury at death It was assumed that the toxicity of the supplement was responsible for the death of the animals Apparently, Handler and Follis had a similar experience with thiouracil but the time at which their animals died was not specified At a level of only 0 05 per cent of propylthiouracil its toxic effect was suffic to permit three of the animals to survive the

TABLE 3

COMPOSITION OF BASAL DIET

Dietary ingredient	Per cent
Yeast (Fleischmann's active dried baker's yeast)	15
Cornstarch	60
Lard	5
Salt mixture*	3
Sugar vitamin mixture**	1
Cod liver oil (Mead Johnson Company 3000 I U of Vitamin A and 400 I U of Vitamin D per gram)	2
Celluflour	2

* Hegsted's salt mixture (Personal communication for details see Abelt et al [illegible])

** The sugar vitamin mixture contributed the following amounts of B vitamins per 8 gm. of food thiamin hydrochloride 20 μgm riboflavin 25 μgm pyridoxine hydrochloride 20 μgm calcium pantothenate 100 μgm

TABLE 4

THE EFFECT OF DIETARY SUPPLEMENTS ON THE DEVELOPMENT OF LIVER NECROSIS

Dietary supplement	Rats dying of necrosis or killed in extremis		Rats dying of other causes		Total incidence of necrosis corr d.*	
	Number rats	Av. days on diet	Number rats	Av. days on diet	Number	%
None	9	39	1	42	9/10	90
0.3% desiccated thyroid	7	20	3	13	7/8	88
0.05% propylthiouracil	2	59	5	55	3/10**	30
0.3% potassium thiocyanate	8	56	2	69	8/10	80
0.3% desiccated thyroid +0.5% L-cystine	0	—	8	57	0	0
0.3% desiccated thyroid +2 mgm α tocopherol acetate per 8 gm diet	0	—	9	43	0	0

From McLean and Beveridge [illegible]

* Rats that died of causes other than liver necrosis were eliminated from consideration if they died prior to the time at which the first death due to liver necrosis occurred in the remainder of the group. This convention had to be applied in the case of the group receiving 0.3% desiccated thyroid.

**One animal dying at 60 days showed necrosis in a small part of one lobe of the liver and it was believed that this lesion was insufficient to have caused the death of the animal.

experiment, and of the animals that died only three showed acute liver damage The incidence of [illegible] therefore [illegible] time to develop [illegible] propylthiouracil [illegible] the basal diet [illegible] antithyroid action of the drug, the possibility that the prophylactic action may be due to the provision of sulphydryl groups must be kept in mind since György [illegible] have shown that [illegible]

Acc[illegible] those [illegible] can be [illegible] primary action is to depress the utilization of iodine in the synthesis of thyroxine. Propylthiouracil may be considered to be a member of the latter group and it was considered worth while to test the effect of a compound belonging to the former class. Potassium thiocyanate was chosen and, at a level of 0.3

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The diet used by these investigators contained 0 2 per cent desiccated thyroid and caused acute liver damage in about 50 per cent of 50 gm male rats at an average interval of 40 days compared to an incidence of 100 per cent in 70 days for similar rats on the basal diet The diet (TABLE 3) used by us[10] contained 0 3 per cent desiccated thyroid and (TABLE 4) caused 88 per cent necrosis after an average interval of only 20 days or half the time required by the basal diet 39 days These results are indicative of a profound effect of thyroid tissue on the development of liver damage The effect of feeding propylthiouracil on the other hand is not so clear cut With a level of 0 3 per cent propylthiouracil the rats died at an average interval of 47 days but they showed no evidence of hepatic injury at death It was assumed that the toxicity of the supplement

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Cellufiour	2

* Wesson's salt mixture (Personal communication for details see Abell et al [1])

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[illegible]

antithyroid action of the drug, the possibility that the prophylactic action may be due to the provision of sulphydryl groups must be kept in mind since György et al [11] have shown that this compound exists in tautomeric forms

According to Astwood [illegible]

[illegible] Propylthiouracil may be considered to be a member of the latter group, and it was considered worth while to test the effect of a compound belonging to the former class Potassium thiocyanate was chosen and, at a level of 0 3

per cent, it effected a significant increase in the length of time required for death to occur, 56 days compared to 39 days for the rats on the basal diet. There was no effect, however, on the actual incidence of acute hepatic damage.

Supplementation of the basal diet with 0.5 per cent L-cystine or 2 mgm alpha tocopherol per 8 gm of diet did not overcome the well known toxic effects of excessive amounts of thyroid tissue, but the animals survived beyond the average time at which death occurred on the unsupplemented ration (indeed, three rats survived the experimental period of 105 days), and none of the rats in these groups developed massive hepatic necrosis. The fact that both alpha tocopherol and cystine prevented liver necrosis in hyperthyroid animals led us to conclude that a high metabolic rate *per se* is not a prime factor in pathogenesis and that the level of thyroid activity is a subsidiary, not a determining factor, in the production or prevention of this hepatic lesion.

From time to time, we have observed pancreatic necrosis in some of the rats killed *in extremis* and showing acute liver damage. This finding raised the possibility that, despite the absence of any consistent obvious lesion in the pancreas, this organ might be in some way involved in the process under study. An initial pancreatic lesion might result in active proteolytic enzymes gaining the portal blood stream and causing the type of extensive liver damage under investigation here. Certainly, the intravenous administration of chymotrypsin as was shown by Tagnon *et al.* causes wide spread damage including necrosis, not only of liver cells, but also of kidney tubules, a lesion that is also frequently seen in animals showing acute massive hepatic necrosis. The fact that the blood from the pancreas travels primarily to the left half of the liver fitted in quite nicely with the well substantiated observation that the left half of the liver is usually the most severely damaged part. It was decided, therefore, to investigate the possible etiological role of the pancreas although it must be admitted that our thoughts were directed primarily towards the exocrine rat' than the endocrine function of this gland.

As complete a pancreatectomy as possible was performed on a number . males rats. Since a splenectomy was done in this procedure, control groups c rats were comprised not only of sham-operated animals, but also a group o which splenectomies had been carried out. These animals, of average initial weight about 140 gm, were fed a basal necrogenic diet in the usual way and after an interval of 290 days the experiment was terminated. The results are shown in TABLE 5.

TABLE 5

EFFECT OF PARTIAL PANCREATECTOMY AND SPLENECTOMY ON THE DEVELOPMENT OF LIVER NECROSIS

Group	Number of rats showing liver necrosis	Incidence of necrosis	Days on diet of rats showing liver necrosis	
			Average	Range
Normal controls	1	1/11	68	
Depancreatized and splenectomized	3	3/10	181	143–213
Splenectomized	3	3/11	195	176–213

Although we were somewhat disturbed by the low incidence of liver necrosis these findings show clearly that neither partial pancreatectomy plus splenectomy, nor splenectomy alone has any effect on the development of acute liver damage On the other hand we readily admit that there may have been sufficient pancreatic tissue left to account for the production of the liver lesion, but we regard this possibility as being remote

In conclusion it would appear that although various investigations have demonstrated that secretions from the endocrine glands do influence one way or another the development of dietary liver necrosis there is no clear cut evidence to show that any of the endocrine secretions have a primary role in the development of this hepatic lesion

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Discussion of the Paper

Doctor Klaus Schwarz I should like to point out that food intake greatly influences the development of dietary necrotic liver degeneration This variable is of primary importance in the interpretation of results such as reported by Doctor Beveridge We have investigated in collaboration with Doctor R Scow of our Institute the effect of thyroidectomy on the development of this deficiency It was found that Sprague Dawley rats when thyroidectomized at weaning did not develop necrotic liver degeneration even after experimental periods extended long beyond the time of death of all the littermate controls fed *ad libitum* Realizing however that rats fed *ad libitum* do not constitute proper controls for others which do not eat due to some endocrine disturbance we have run simultaneous unoperated littermate controls which were *pairfed to the thyroidectomized group* These animals which received approximately 50 per cent of the diet consumed by the *ad libitum* fed group survived as well as the thyroidectomized ones It may be possible that we have to interpret all the reported effects of decreased or increased thyroid activity on necrotic liver degeneration as resulting from decreased or increased food consumption and not from the endocrine disturbance directly

FACTORS PROTECTING AGAINST DIETARY NECROTIC LIVER DEGENERATION

By Klaus Schwarz

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Introduction

It is a pertinent characteristic of every natural phenomenon that, in ad to its primary cause, secondary conditioning causes influence its origin This is certainly true for all deficiency diseases In the preceding papers, va nonspecific factors affecting the development of dietary necrotic liver degen tion in the rat have been presented and discussed The present pape devoted to the factors which are primarily involved

The development of necrotic liver degeneration has been shown to depe on such complicating circumstances as the over all composition of the deficie diet, the endocrine status of the animal, its daily food allowance, and its e vironmental temperature No matter how profoundly these various influence either enhance or inhibit the process the fact remains that dietary necrotic liver degeneration is, in the first place, a deficiency disease It can be pro- duced experimentally by diets which lack certain essential nutrients and it can be prevented by these dietary components The disease is different how ever, from many other deficiency diseases in that it does not result from the lack of one single dietary factor It is more complicated At least three different nutritional factors must be lacking simultaneously before the disease develops This complexity has prevented a quick solution of the many problems which are presented by the deficiency It also makes dietary necrotic liver degenera tion especially interesting from a biochemical and metabolic point of view Like fatty liver and cirrhosis, the other liver disease produced by dietary means dietary necrotic liver degeneration is of multiple origin, but the nutritional fac tors involved are different Substances which prevent the first syndrome can even enhance the development of the second, and vice versa [1]

The three nutritional factors at present clearly separable and recognized as protecting against dietary necrotic liver degeneration are cystine, vitamin E and Factor 3 The action of cystine was detected in 1935 by Weichselbaum and later by Daft Sebrell and Lillie,[2] it was confirmed by Hock and Fink[4] and many others The role of vitamin E as a protective agent was elucidated between 1941 and 1944 by Schwarz [5 6*] and has been confirmed by Gyorgy and collaborators[7] and by various other groups between 1947 and 1950 Fi nally the existence of Factor 3 has been established in this laboratory only a few years ago [8] An account of this work is given below

Diets for the Production of Dietary Necrotic Liver Degeneration

Rations designed to produce dietary necrotic liver degeneration must be low in cystine, free from vitamin E and, furthermore, deficient in Factor 3, since

* Printed and published in 1945 in the United States under the ausp ces of U S O A P C by J W Edwards Ann Arbor M ch.

TABLE 1
DIETS PRODUCING NECROTIC LIVER DEGENERATION IN RATS

Cystine free (corn starch 80%, dry milk powder 16%)	Weichselbaum[2]
Cystine free (amino acids in crystalline form)	DuVigneaud Dyer, and Kies[24] Glynn Himsworth, and Neuberger[16]
Low casein (10% or less)	Daft Sebrell and Lillie[9] Gyorgy and Goldblatt[11] Hove and Harris[25] Schwarz[5]
Casein VI (alkali treated, 15%)	Hock and Fink[4]
Yeast	Glynn and Himsworth[15] Schwarz[8, 20] Gyorgy and Goldblatt[26] Schwarz[27]
High cod liver oil	
Potato protein Pea protein Cereal protein Gelatin } in various combinations	Hock and Fink[28]
Soybean meal (raw, fat extracted)	Matet Matet and Fridenson[29]
Soybean protein	Olson[30]

TABLE 2
COMPOSITION OF DIETS

Casein VI diet		*Torula* yeast diet	
	Parts		%
Casein VI*	15	Torula yeast	30
Butter fat (extracted)*	5**	Lard, Vit E-free††	5
Sucrose	65	Sucrose	59
Salts***	5	Salts‡	5
Vitamins supplemented separately†		Vitamin powder‡‡	1

* Preparation described in Schwarz[8]
** *In earlier experiments 10 parts*
*** For composition see Schwarz[5, 8]
† See Schwarz[6]
†† Vitamin E free animal fat stripped by molecular distillation (Distillation Products Division Rochester New York)
‡ Composition same as salts in Casein VI diet since April 1951 Hubbel Mendel and Wakeman J Nutrition 14 273 (1937)
‡‡ Composition see Schwarz[8]

sulfur amino acids in respect to dietary necrotic liver degeneration It is *generally accepted that methionine and not cystine is the sulfur-containing* amino acid essential for growth and for well being of animals It is quite evident that the rat forms cystine from methionine, whereas it is unable to form methionine from cystine The requirement for sulfur amino acids can be satisfied by methionine alone but not by cystine[14] In dietary necrotic liver degeneration, on the other hand we have a case where cystine is more important than methionine, at least in the survival of the animal Cystine *affords protection*, whereas *methionine* is much less effective This was discovered in 1935 by Weichselbaum[2] and was later demonstrated by Himsworth and collaborators, who at first considered necrotic liver degeneration to be a methionine deficiency[15] and then upon more careful investigation, asserted that

TABLE 3
COMPARISON OF PROTECTIVE EFFECTS OF L-CYSTINE AND L-METHIONINE AGAINST DIETARY NECROTIC LIVER DEGENERATION

Supplement	%	% protection*
L-cystine	0 1	17
L-methionine	0 123	3
L-cystine	0 2	68
L-methionine	0 246	12

* Per cent protection = per cent reduction of reciprocal survival time as compared to litter-mate control group

not methionine, but rather cystine, is the protective agent [16] When L-cystine and L-methionine are compared at different dose levels in the Torula diet it is seen that supplements of L-methionine are not entirely ineffective They show usually about 25-35 per cent of the activity of equivalent amounts of L-cystine A representative experiment is given in TABLE 3

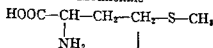
Teleologically, it could be theorized from these results that in an animal succumbing to liver necrosis, the transformation of methionine to cystine is not sufficient to prevent death If this were a metabolic insufficiency it could be due either to an increased demand for cystine exceeding the natural tendency and capacity to perform this conversion or to a real inhibition of the transformation by a metabolic block which could be caused by lack of vitamin E and Factor 3 It is conceivable, but requires further elucidation that the methionine-cystine transformation occupies a key position in the antagonistic relationship between the factors which protect against dietary necrotic liver degeneration (prevented by cystine) on the one hand and the factors which protect against fatty liver and cirrhosis (prevented by methyl groups) on the other The pathway of the transformation as it is understood at present involves the main reactions found on the following page

In an attempt to detect which step of the transformation is inadequately performed, various intermediates were fed DL homocystine was as ineffective as methionine Cystathionine was not tested The last step in the conversion of methionine to cystine is the oxidation of cysteine to cystine It was found, to our surprise, in repeated experiments that L-cysteine when fed either by stomach tube or when given in the diet does not replace L cystine The activity of cysteine is as low as that of methionine (TABLE 4)

From these results a number of essential questions can be derived One might ask whether cysteine is as easily converted to cystine in normal intermediary metabolism, as is usually taken for granted or whether in animals on necrosis producing diets this oxidative process is especially defective The question also arises whether vitamin E or Factor 3 participates catalytically in this reaction or in some other closely associated reaction of sulfur amino acid metabolism Furthermore, it becomes a question as to whether the effect of cystine on dietary necrotic liver degeneration is strictly a physiological nutritional one or whether cystine in this instance exerts some pharmacological action in excess of its normal biological functions and in excess of the normal cystine requirement It deserves attention that the effect of cystine

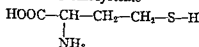
Methionine Cystine Transformation

Methionine

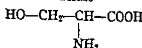




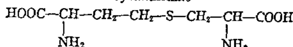
Homocysteine



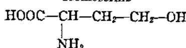
Serine



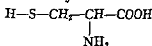
Cystathionine



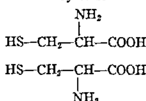
Homoserine



Cysteine

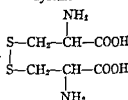


2 Cysteine





Cystine



is obviously not due to a known detoxification mechanism for the simple reason that in detoxification reactions cysteine is as active as cystine [17]

Vitamin E as a Protecting Agent

After two years highly purified fractions [illegible] were obtained The best concentrates contained up to 44 per cent of a substance which was shown to be vitamin E Early in 1943 synthetic α tocopherol acetate was found to be active

From a series of tests performed in 1943 the daily dose of synthetic α tocopherol acetate required for 50 per cent protection was found to be 67 γ Using

TABLE 4

COMPARISON OF L-CYSTINE AND L-CYSTEINE (SUPPLEMENTATION STARTED ON THE 25TH DAY)

Supplement	%	% protection*
L-cystine	0.5	96.6
L-cysteine	0.5	16.5
L-cystine	1.0	97
L-cysteine	1.0	26.2

* Per cent protection = per cent reduction of reciprocal survival time as compared to alternate control group.

the Torula yeast diet and Sprague Dawley rats in Bethesda in 1950 we found that about 50 γ are required daily for the same effect. In the later experiments the vitamin was added to the diet. These levels of vitamin E required were in the earlier publications considered to be much higher than the normal vitamin E requirement. Dietary necrotic liver degeneration was interpreted as a 'relative' vitamin E deficiency.[8] In view of more recent data, however, it appears that these quantities of vitamin E are actually well within the range of the normal vitamin E requirement for the prevention of deficiency symptoms in rats. It has, for example, been reported that between 30 and 70 γ of synthetic α tocopherol acetate are required daily for maintenance of normal seminiferous epithelium in growing rats.[18] In the light of these observations dietary necrotic liver degeneration appears to be a form of vitamin E deficiency which is modified by the simultaneous absence of Factor 3. The occurrence of dietary necrotic liver degeneration as a vitamin E deficiency indicates strongly that tocopherol has functions which are of basic importance for normal intermediary metabolism.

Factor 3 against Dietary Necrotic Liver Degeneration

An attempt was made in 1943–1944 to determine whether the procedure used for the preparation of casein VI from Hammarsten casein caused a toxic component to be formed or whether a protective factor was removed. Evidence for the first possibility could not be found and some evidence for the later was obtained. Since Hammersten casein, from which the casein VI was produced, is suitable for the production of vitamin E deficiencies, the protective substance removed could be assumed to be different from tocopherol. Analyses for cystine and methionine showed that the difference between the two caseins could not be ascribed to differences in cystine or methionine (TABLE 5).

The reasoning which led to the discovery of Factor 3 may be further illustrated by the following example. Two diets are listed in TABLE 6. One is a conventional 15 per cent 'vitamin free' casein diet, the other a 30 per cent Torula ration (approximately 14 per cent protein). The first does not produce dietary liver necrosis. The other one, prepared with the same dietary ingredients except for the substitution of Torula yeast at the expense of casein and of sucrose, produces the damage with 100 per cent incidence. When the ingredients used in this experiment were analyzed colorimetrically and microbiologically for cystine and methionine, it became evident that there was no profound

TABLE 5
S-AMINO ACIDS IN CASEIN DIETS

	Cystine	Methionine	Incidence of dietary necr liver degenerat
	%	%	%
Casein Hammarsten diet (15%)	0 06	0 52	0
Casein VI diet (15%)	0 00	0 54	84

TABLE 6
S-AMINO ACIDS IN TORULA DIET

	Cystine	Methionine	Incidence dietary necr liver degenerati
	%	%	%
Control diet, 'Vitamin free' casein (15%)	0 075	0 45	0
Torula yeast diet (30%)	0 29	0 38	100

and decisive difference in sulfur-containing amino acids The Torula diet w even somewhat superior in cystine, but the differences were altogether so sm that they were, in view of the relatively high levels of cystine and methioni required for protection, not of significance Accordingly, the fundamental di *similarity between these two rations in regard to liver necrosis could not b* attributed to differences in cystine or methionine Moreover, the dissimilarit could not be caused by a difference in the vitamin E contents, since bot rations were apparently practically free from tocopherol Both "vitamin free casein, on the one hand, and dried yeast, on the other, are standard ingredient of diets producing vitamin E deficiencies of the conventional type From thes considerations, it became evident that the difference between the two diet could be expected to be due to some other factor, and it seemed logical to tes the "vitamin free" casein for liver protecting activity in the Torula assay (FIGURE 1) Addition of 3 per cent of the "vitamin free" casein produced a reduction of 66 per cent in reciprocal survival time By comparison, an aminc acid mixture simulating the composition of the casein[19] was without significant effect

The detection of Factor 3 was facilitated by a peculiar discrepancy between European and American yeasts In vitamin E-free diets, almost all European *dried yeasts led to the deficiency*[20] whereas almost all American yeasts, particu larly American brewers' yeasts, did not produce the disease [9, 21] However, an American Torula yeast which is grown commercially on a rather simple medium was found, in 1949, to produce the deficiency with 100 per cent incidence[9] Since that time the 30 per cent Torula diet has been used in our laboratory in extensive investigations in attempts to concentrate and identify Factor 3

TABLE 7

So far, n

offer pro

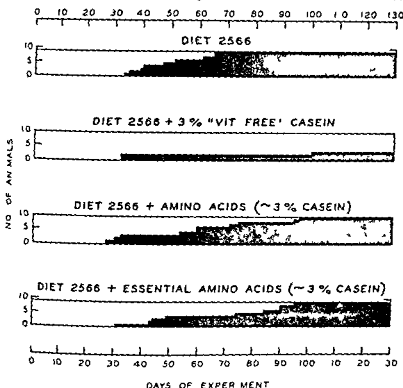


Figure 3 Prevention of death from dietary liver degeneration by 3 per cent casein (Black areas Animals dead from necrotic liver degeneration)

why the disease could not be produced in numerous attempts with diets based mainly on these yeasts

As starting material for the purification for Factor 3 a variety of natural sources can be used. Factor 3 has in common with most other essential accessory foodstuffs, the property of being strongly bound to proteins It cannot be liberated by ordinary extraction procedures Therefore most of the sources of Factor 3 have to be acid hydrolyzed before the fractionation of the active principle can begin Acid hydrolysis does not alter the total Factor 3 activity since the active substance is quite stable against this treatment Factor 3 is found in free form in enzymatic protein digests A commercial casein preparation has been used for the large scale purification of the active substance The first three steps of [illegible] had [illegible] been described elsewhere [illegible] The active

TABLE 7
PROTECTIVE EFFECT OF YEAST K

Torula yeast %	Supplements	No of A animals	Dead due to liver degeneration	Avg of reciprocal survival time (X 100)	% protection*
30	—	9	9	2 40 ± 18	—
27	Yeast K 3%	10	2	58 ± 25	76
25	Yeast K 5%	8	0	00 ± 0	100

* Per cent protection = per cent reduction of reciprocal survival time as compared to alternate control group

a small volume of water From this fraction, a heavy precipitate is formed upon standing in the cold, which contains most of the Factor 3 activity Further steps of purification involve treatment with solvents like ethanol, butanol, and phenol, and also precipitation reactions with phosphotungstic acid and barium hydroxide In this manner, fractions can be obtained from casein which protect the animals from dietary necrotic liver degeneration when given in very small amounts

since the customary reactions known to destroy these substances do not inactivate our fractions Factor 3 can be expected to have a rather low molecular weight It might well be that it is identical with a compound which is already known, though a large number of substances occurring in nature have been tested and found to be inactive

Metabolic Interrelations in Dietary Necrotic Liver Degeneration

The peculiar fact that, in dietary necrotic liver degeneration, three sub

and Factor 3, on the other hand, may be remains to be clarified The quantitative proportions between these substances have to be elaborated when all of them are available in pure form Considering the fact, however, that between 400 to 800 molecules of cystine are required to produce as much protection as that offered by one molecule of α tocopherol, it could be postulated that the role of vitamin E is of a catalytic nature whereas cystine or a cystine derivative functions as a substrate[22] From the dosage levels of Factor 3 required to obtain comparable degrees of protection, it can be suggested that ... all belong to the group of substances which have catalytic

the dietary relationship described

An attempt has been made to ...

...of the disease, lac...

In preliminary ... the
...ner of animals on ... producing diet was greatly increased upon
injection of Na monofluoroacetate For weeks prior to succumbing to liver necrosis, animals on necrogenic diets show a serious incompatibility for fat [10] These and other findings make it conceivable that the area of the primary metabolic breakdown in dietary necrotic liver degeneration is in the citric acid cycle or in some closely related function

Summary

Three different nutritional factors have been recognized to be specifically connected with dietary necrotic liver degeneration cystine vitamin E and Factor 3 Diets for the production of the deficiency in rats must be low in cystine and deficient in vitamin E and in Factor 3 Two rations fulfilling these requirements are described the casein VI diet, used between 1940–1945 and the 30 per cent Torula yeast ration, in use since 1949

In the prevention of dietary necrotic liver degeneration cystine is required in rather large amounts (0 2 1 per cent in the diet) Other S containing amino acids, like methionine, homocystine and also cysteine are only approximately one third as effective as cystine

α-Tocopherol acetate was found to afford 50 per cent protection at 67 γ daily per animal in the old series and about 50 γ daily in the new series These levels are within the range of the normal vitamin E requirement of the rat

The detection of Factor 3, its occurrence in various caseins and in brewers yeast and the method of purification of Factor 3 concentrates are described Factor 3 is of low molecular weight, stable against acid hydrolysis water soluble and apparently not identical with any of the presently well known vitamins or amino acids

The possible metabolic interrelations between these factors and also the metabolic aspects of dietary necrotic liver degeneration are discussed ...
preliminary results it appe...
...nctions in the liver ...
...mary metabolic defec...

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ENZYME ABNORMALITIES ASSOCIATED WITH DIETARY NECROTIC LIVER DEGENERATION IN RATS*

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Although acute massive hepatic necrosis in the rat has been shown to be a manifestation of malnutrition, its exact biochemical pathogenesis is unknown Frank tissue necrosis is generally assumed to be preceded by single or multiple biochemical lesions, and it seems probable that this particular syndrome of hepatic necrosis is no exception to the rule The fact that a deficiency of one or more dietary nutrients appears to be related in a causal manner to this syndrome was of particular interest to us and led us to undertake a study of its biochemical evolution It was hoped, in addition that these studies would contribute to the elucidation of the interrelationships between the sulfur amino acids and vitamin E, both of which are protective against this disease Our initial approach to this problem was to compare the content of selected enzymes and coenzymes in the livers of rats in which hepatic necrosis was produced with others which were protected by suitable dietary supplements The results though preliminary, are consistent with the view that acute massive hepatic necrosis in the rat is associated with differential disruption of the citric acid cycle and depressed tissue levels of coenzyme A

Male weanling rats of the Sprague Dawley strain were used in these experiments One group was fed the basal diet composed basically of soy protein and l d l ... TABLE 1

devoid of

t plus pro

tective supplements These included Vitamin E alone cystine alone cystine plus Vitamin E, methionine alone, and methionine plus vitamin E Vitamin E was administered at the level of 3 mgm of α tocopherol twice weekly by dropper and the ... he le el of 0 5 per cent

lated intermediate growth rates between that observed on the basal diets and the diet containing 18 per cent casein

After 4 to 6 weeks, animals on the basal diet developed lesions typical of acute massive hepatic necrosis both grossly and microscopically [1] No lesions were observed in the rats receiving vitamin F or methionine Occasional

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At the time of the first deaths in the basal group rats were taken from all groups for the determination of hepatic pyruvic oxidase, succinoxidase, trans-

* Supported in part by grants in aid from the Nutrition Foundation Inc New York N Y and the Williams Waterman Fund of the Research Corporation New York N Y

TABLE 1
BASAL DIET

	Per cent
Alpha protein	15 0
Sucrose	72 8
Lard	6 0
Salts (H M W)	4 0
Cod liver oil	2 0
Choline	0 2

B vitamins were added in the following amounts expressed as mgm per kgm diet Thiamine 5 0 riboflavin 5 0 pyridoxine 5 0 calcium pantothenate 20 0 niacin 40 0 folic acid 1.5 and biotin 0 05 Menadione was added at the level of 0 08 mgm./kgm

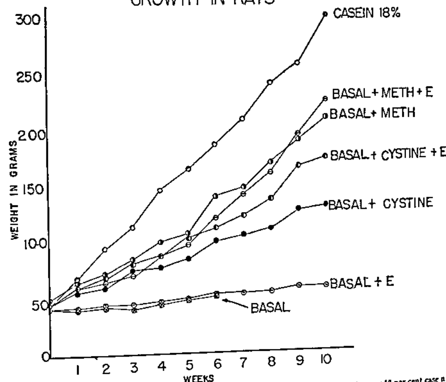


FIGURE 1 Growth rates of weanling rats fed the experimental diets and a diet containing 18 per cent casein over a 10-week period.

aminase and xanthine oxidase These particular enzymes were chosen for assay in order to gain as much information as possible about the carbohydrate amino acid and purine metabolism of necrotic and protected livers The animals were killed by decapitation and 10 per cent homogenates of liver in isotonic KCl were prepared Appropriate amounts of homogenate were taken for each of the assays

The term pyruvic oxidase is used in this paper in its broadest sense of includ

TABLE 2
PYRUVATE OXIDATION IN HOMOGENATES OF LIVER FROM NECROTIC NON NECROTIC DEFICIENT VITAMIN E- AND SULFUR AMINO ACID-TREATED RATS

Diet	Rats nr	Necrosis gross	Qo_2 none	Qo_2 pyruvate	Qo_2 pyruvate + ATP
Basal	9	+	1 0 ± 0 1	1 5 ± 0 3	3 1 ± 0 6
Basal	5	0	2 2 ± 0 6	2 8 ± 0 4	7 2 ± 0 9
Basal + E	6	0	2 9 ± 0 6	3 2 ± 0 8	6 9 ± 0 9
Basal + cystine	5	0	3 2 ± 0 7	4 8 ± 0 5	6 0 ± 0 9
Basal + cystine + E	5	0	4 0 ± 0 4	5 7 ± 0 7	8 9 ± 1 3
Basal + meth.	4	0	3 3 ± 1 0	3 2 ± 1 1	7 9 ± 0 8
Basal + meth. + E	5	0	3 1 ± 0 4	4 2 ± 0 4	8 8 ± 0 8

. .

(

... added substrate with sodium pyruvate added at 5 mM/liter, and with pyruvate at 5 mM/L, plus ATP at 1 mM/L The addition of ATP to the last flask permitted the observation of pyruvate oxidation under conditions which tended to prevent deactivation of tricarboxylic acid cycle intermediates and the fission of coenzymes Succinoxidase was determined by the method of Schneider and Potter[3] transaminase by the method of Ames and Elvehjem[4] and xanthine oxidase by the method of Axelrod and Elvehjem[5] All substrates were tipped in at "zero' time The units for the pyruvic oxidase assay are given as Qo_2, calculated for a 20-minute period and the units for the other assays are those used by their respective authors

The results obtained with the pyruvic oxidase assay are shown in TABLE 2 The animals on the basal diet were divided into two groups depending upon the presence or absence of gross necrosis It can be seen that both the endogenous O_2 uptake and the oxidation of pyruvate, with or without added ATP was markedly reduced in homogenates of liver from rats showing gross necrosis The activity of the control group (rats fed basal plus methionine plus vitamin E)...

from groups fed the basal diet plus vitamin E were intermediate between the rats on the basal diet not showing necrosis and the controls although not significantly different from either None of the rats on other protective regimens showed values significantly different from the controls

TABLE 3 presents the results of the assays for succinoxidase transaminase and xanthine oxidase in these same groups of animals It is noteworthy that the activity of succinoxidase and transaminase are virtually the same in all groups The ...
is statistically
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in comparison with the drop in pyruvic oxidase activity It seemed remarkable to us that hepatic transaminase activity was maintained in the severely

TABLE 3

SUCCINOXIDASE, TRANSAMINASE, AND XANTHINE OXIDASE ACTIVITIES OF LIVER FROM NECROTIC, NON NECROTIC-DEFICIENT, VITAMIN E- AND SULFUR AMINO ACID-TREATED-RATS

Diet	Rats no	Necrosis gross	Succinoxidase	Trans-aminase	Xanthine oxidase
Basal	9	+	31 ± 3	64 ± 2	39 ± 9
Basal	5	0	38 ± 4	55 ± 5	13 ± 9
Basal + E	6	0	39 ± 3	60 ± 4	6 ± 5
Basal + cystine	5	0	39 ± 2	57 ± 4	137 ± 8
Basal + cystine + F	5	0	47 ± 5	65 ± 9	20 ± 13
Basal + meth	4	0	40 ± 2	63 ± 11	160 ± 10
Basal + meth + E	5	0	42 ± 2	62 ± 8	138 ± 8

necrotic group, particularly since it has been reported that certain manifestations of pyridoxine deficiency appear in association with vitamin E deficiency in rabbits [6,7]

The xanthine oxidase activity of the livers from animals in this study were of great interest The lowest values were obtained in rats fed the basal diet plus vitamin E This was *probably due to the poor biological value of the* [illegible]

oxidase This effect of vitamin E lack mirrors an effect seen in rabbits deprived of vitamin E, in which the hepatic xanthine oxidase becomes markedly elevated [9] The tendency of vitamin E to depress the liver xanthine oxidase is also demonstrated by a comparison of the liver xanthine oxidase values of the *groups given cystine alone and cystine plus vitamin E* (TABLE 3) When methionine is the supplement, the vitamin E effect is abolished Whether this *vitamin E effect is dependent upon adequate amounts of molybdenum* (which is abundant in soy protein) in the diet,[10,11] remains to be determined If methionine is required for the elaboration of xanthine oxidase in the liver these effects of vitamin E might be explained on the basis of a decreased utilization of methionine for other purposes in vitamin F deficiency The effect of cystine would be interpreted as a sparing action

[illegible] occurred with acute

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decline of the activity of all the cellular enzymes [illegible] es, in addition to catabolic ones known to increase with cell lysis such as the cathepsins, desamidases, phosphatases, and nucleases, may *even increase* *How* much of this change can be traced to the underlying pathological process preceding necrosis and how much is a concomitant of necrosis *per se?* The disruption of some portion of the citric acid cycle exclusive of the succinoxidase system seemed out of proportion to the other changes and it was *thought* profitable to explore the status of certain catalysts in that sequence of reactions There were obviously many possibilities The observation *that the*

TABLE 4

CONTENT OF COENZYME A IN LIVER FROM NECROTIC NON NECROTIC-DEFICIENT VITAMIN E TREATED, AND CONTROL RATS

Diet	Rats nr	Necros s gross	Coenzyme A un ts/gm
Basal	9	+	37 ± 5
Basal	5	0	68 ± 9
Basal + E	4	0	92 ± 8
Control	4	0	180 ± 20

rats on the basal diet developed the "porphyrin whiskers usually associated with pantothenic acid deficiency led us to determine the rates of acetylation of para aminobenzoic acid in intact deficient rats The finding that these were markedly depressed in the animals with liver necrosis and moderately depressed in deficient animals without necrosis[12] coupled with the realization that coenzyme A, the active form of pantothenic acid in tissues and the bearer of the C₂-fragment, contained a sulfur moiety[13] not unrelated to cystine, stimulated us to make direct determinations of coenzyme A in livers from these animals The technique of assay was that of Kaplan and Lipmann[14] and the results are shown in TABLE 4

The rats having liver necrosis had markedly depressed values for hepatic coenzyme A Those on the deficient diet not showing necrosis had levels somewhat higher, but even these were as low as ordinarily produced by pantothenic acid restriction alone[15] Those rats protected against necrosis with vitamin E had still higher values and control rats fed either chow or the 18 per cent casein diet had high values which were similar to other normal rats examined in the past Because of this relationship between coenzyme A content and the suspected degree of hepatic disability in this dietary disease,

Hypothesis

Is the depression of coenzyme A in necrotic liver tissue specific for this dietary deficiency disease, or does it represent a generalized result of necrosis? The fact that transaminase and xanthine oxidase activities which are also dependent upon coenzymes (pyridoxal phosphate and flavin adeninedinucleotide) are well maintained, and the fact of the low coenzyme A content of non necrotic deficient liver tissue suggest that the coenzyme depletion of necrotic liver tissue is not nonspecific It will be important however, to make direct determination of other coenzymes in the livers of deficient rats and these studies are in progress

Is β mercaptoethylamine, the sulfur moiety of coenzyme A derived from dietary cystine? One can visualize a reasonably simple pathway from cystine to β-mercaptoethylamine via one step of reduction and one step of decarboxylation although the order of the steps and all of the enzymes involved are not known Studies with S^{35} labeled cystine are in progress to verify this relationship and a search for the probable enzymes involved is under way

Finally, the possible role in hepatic necrosis of another sulfur containing coenzyme in the metabolism of pyruvate α lipoic acid must be ruled out[16] Although this coenzyme is normally synthesized by mammalian tissues it is conceivable that rigid organic sulfur restriction in the diet may also limit its synthesis

The conclusions which can be drawn from this preliminary study are necessarily subject to qualification. I believe it can be said however that rat liver rendered necrotic by dietary restriction of cystine methionine and vitamin E presents an enzyme profile which features a defect in the oxidation of pyruvate which does not involve all enzymes of the citric acid cycle It may be said furthermore that dietary restriction of the sulfur amino acids results in a lowering of coenzyme A equivalent to that seen in marked pantothenic acid deficiency prior to the onset of hepatic necrosis and suggests that a nonvitamin precursor of a tissue coenzyme may be as important in the elaboration of the coenzyme as the vitamin itself Vitamin E protects against hepatic necrosis tends to preserve the coenzyme A level and appears to divert dietary methionine into channels other than the synthesis of xanthine oxidase

Acknowledgments

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Discussion of the Paper

DOCTOR KLAUS SCHWARZ The results of Doctor Olson and his collaborators are in harmony with some of our own Investigations in our laboratory have shown that the site of the primary metabolic defect in the dietary necrotic

liver degenerat

pantethine (*Lactobacillus bul*
activity against dietary nec
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References to the Discussion

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THE XANTHINE OXIDASE FACTOR (MOLYBDENUM)*

By W W Westerfeld and Dan A Richert

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The effect of diet on the xanthine oxidase activities of various rat tissues was studied as a sequel to an earlier investigation in which it was observed that

systems that could theoretically be concerned with acetaldehyde metabolism, the chemically similar xanthine and aldehyde oxidases seemed most likely to be dependent upon a dietary factor not already incorporated in the purified rations used Since methods for studying xanthine oxidase were available, our attention was directed to a study of the relationship between tissue xanthine oxidase in rats and the dietary factors supplied to the animals

Xanthine oxidase in rat tissues At the time this work was started, riboflavin[2] and protein deficiencies[3] had been reported to decrease the concentration of liver xanthine oxidase in the rat Our early studies were directed toward determining (1) the distribution of xanthine oxidase in rat tissues and (2) the

or similar to that shown in TABLE 1 Low protein diets were prepared by replacing all or part of the casein with additional glucose

The results of these studies[4] with adult rats are shown in TABLE 2 Small amounts of the enzyme were found in lung, kidney, spleen, and skin, but only

amount of enzyme was present in the liver while 20 per cent was ... skin and small intestine Feeding a low protein diet removed at least 70 per cent of the total enzyme from the rat, but the amount remaining was adequate to maintain a normal excretion of uric acid and allantoin [5] This deserves some emphasis because it indicates that either (1) uric acid can be formed by a mechanism not involving xanthine oxidase or (2) the amount of xanthine oxidase normally present in the rat far exceeds the amount required to fulfill its usual function *In vitro* studies[6] could not detect any metabolic pathway for the formation of uric acid other than the one utilizing xanthine oxidase The remaining conclusion is that rat tissues normally have at least a three fold

* The studies reported herein were supported by grants from the Nutrition Foundation the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and the Office of Naval Research

TABLE 1

COMPOSITION OF THE PURIFIED DIET USED IN STUDIES OF THE XANTHINE OXIDASE FACTOR

	gm		mgm
Casein (Labco)	24	Choline chloride	100
Crisco	4	Nicotinic acid	2 5
Wessen oil	2	Calcium pantothenate	1 0
Cod liver oil	1	Riboflavin	0 4
Salts (Phillips and Hart)*	4	Thiamine	0 4
Glucose	65	Pyridoxine	0 4

*P & H salts contain Na K Ca Mg Fe Mn Zn Cu Cl I phosphate sulfate carbonate and citrate

TABLE 2

THE EFFECT OF FEEDING A PURIFIED 8 PER CENT CASEIN DIET ON THE XANTHINE OXIDASE ACTIVITIES OF ADULT RAT TISSUES

	Liver	Sm Int	Kidney	Lung	Spleen	k n
Normal rats (on chow)	41	33	4	9	12	4
8% casein diet (4–28 weeks)	2*	6*	5	8	15	—
Normal (%) distribution	55	20	1	1	2	21

XO act vities recorded as c mm O_2 per 20 minutes per 283 mgm fresh tissue At least 70 per cent of XO n ent re rat is lost on a low protein diet

* The usual determinat on gives values of 0 and 3 for liver and intestine respect vely The recorded values represent the "true" amount present in relation to the normal values (see sect on on determinat on of xanth ne oxidase)

reserve of this enzyme Variations in tissue enzyme concentration or activity cannot automatically be interpreted as a corresponding variation in function

Liver and intestinal xanthine oxidase The weanling rat responded to low protein and other dietary changes more rapidly than the adult rat but other

containing adequate protein and riboflavin allowed slo[illegible] inter-mediate levels, but did not permit the attainment of normal values The incorporation of liver in a diet containing inadequate protein did not modify the protein deficiency depletion of liver xanthine oxidase When the livers were first depleted of xanthine oxidase by a low protein diet the resumption of an adequate protein intake also restored the liver xanthine oxidase to about 75 per cent of the normal level

It was concluded that the liver xanthine oxidase concentration depended

could not be identified with any known nutritional substance, and [illegible] to as the liver residue factor or the xanthine oxidase factor (XOF) The magnitude of the effect of the xanthine oxidase factor on the concentration of liver xanthine oxidase was not large, but it was detectable and measurable

Numerous successful tests were conducted, using the liver xanthine oxidase response as the criterion for the presence of this unidentified factor in the diet, but it was a difficult test to use as a routine assay procedure

The source of XOF for the 75 per cent restoration of liver xanthine oxidase on a purified diet was unknown In retrospect, it may have been derived from the bones The relatively small effect of an XOF deficiency on liver XO could also be due to the availability of bone molybdenum for this response The "dominant" effect of protein in controlling the level of liver xanthine oxidase may be an artifact because it might not be possible to produce a severe deficiency of molybdenum in the liver so long as preformed deposits exist in bones

The effect of protein deficiency on other selected liver constituents has also [illegible]

xanthine oxidase at weaning by virtue of its previous milk diet Feeding a purified diet which was deficient in either protein or the xanthine oxidase factor [illegible] A diet [illegible] starting [illegible] nd XOF were required simultaneously for this response, and protein was not a dominant factor in the intestinal response as it was for liver XO[15] With adequate protein in the diet, intestinal xanthine oxidase reflected the XOF content of the diet, and a bioassay procedure for XOF was established on this basis[16]

Determination of xanthine oxidase Since all of these results depended upon the determination of xanthine oxidase in rat tissues, the accuracy of the method used in determining this enzyme was studied The results in this and other papers were obtained by the manometric procedure originally described by Axelrod and Elvehjem[2] [illegible] correct values for liver [illegible] does not measure correctly small amounts [illegible] XO Ap- [illegible] deter [illegible] uld be made by determining the activity in the pres[illegible] or the activity could be determined in the usual manner and comparative relationships then calculated from the known and constant methylene blue relationship[10, 18] In contrast to an homogenate, xanthine oxidase appeared to be relatively inert in a slice[19]

[illegible] and XOF deficiencies on liver [illegible] rked "detn" show the re[illegible] ig xanthine oxidase The [illegible] ships calculated from the columns labeled [illegible] values obtained in the presence of methylene blue The true relationship shows

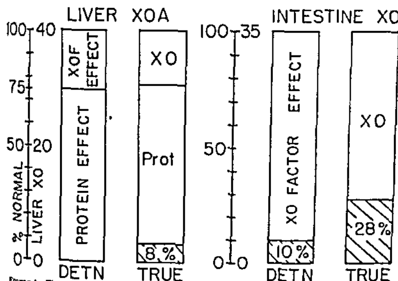


FIGURE 1 The relative effects of deficiencies of protein and xanthine oxidase factor on liver and intestinal xanthine oxidases The XOF effect is obtained in the presence of adequate protein Normal values for liver and intestinal XO were chosen as 40 and 35 (c mm O₂ 20 min /283 mgm fresh tissue respectively Cross hatched areas are residual enzyme not removed by dietary deficiencies

the presence of residual enzyme which cannot be removed by dietary means The magnitude of the XOF effect (in the presence of adequate protein) is obviously much greater for intestine than liver

Xanthine oxidase is not the same enzyme in all species The enzyme in rat liver could be shown to have separate dehydrogenase and oxidase activities by the use of antabuse as an inhibitor of the oxidase portion of the enzyme [20] The enzyme present in bird tissues was identified as a dehydrogenase rather than an oxidase [21, 22] and the effect of dietary variations was shown[23] to be somewhat different for the dehydrogenase in bird tissues as compared with the oxidase in mammalian tissues

Bioassay for XOF ...
culty
casei
diets
aver-
tissue ... e published [6] assay curve proved very satisfactory ... ever, the control levels of intestinal XO increased to 12 or 15 and occasional groups reached 20 Assay curves with such different batches of GBI casein are shown in FIGURE 2 The narrowing of the assay range and the increasing difficulty of the assay procedure as the control level of intestinal XO increased is evident Since it is known that the hot alcohol extraction used for the purification of GBI casein does not remove XOF, the differences recorded in FIGURE 2 are dependent upon chance variation in the purity of the original untreated casein.

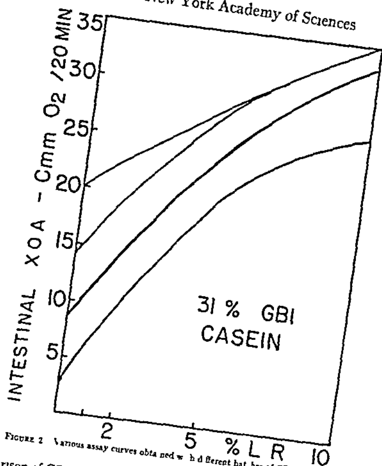


FIGURE 2 Various assay curves obta ned w h d fferent bat hes of GBI case n

A comparison of GBI casein with an acid washed casein Labco (Borden Co) showed the latter to be far superior Control intestinal XO values were 14 and 2 respectively for the two caseins A control diet containing 24 per cent Labco casein has consistently given intestinal XO values of less than 3 ... d the bioassay curve obtained with this casein ... curve shows the rel... in the presence of ... creased the intestina... the lower curve ... id of the tenfold change indicated ... tes due

Diets with 24 and 31 per cent casein yielded ... The 24 per cent ... additional prot... a similar assay ... cent Labco case... ...ore adopted for routine testing ... erratic. The 24 pe...

Xanthine oxidase is apt to be localized in the mucosa of the intestine since muscles are generally free of this enzyme To avoid possible removal of ex posed mucosa by blotting the slit and washed intestine the contents were ex

TABLE 3

ISOLATION PROCEDURE FOR XOF

(1)	[illegible]
(2)	[illegible]
(3)	[illegible]
(4)	[illegible]
(5)	[illegible]
(6)	[illegible]
(7)	[illegible]

and (4) some dark green leafy vegetables Foods which were essentially inactive included (1) muscle meats and fish, and (2) fruits and berries. The substance present in liver residue and foods, which was responsible for this response could not be identified with any factor known to be required in animal nutrition Nor could it be identified with the antithyrotoxic factor present in liver residue [24]

[illegible] XOF [illegible] terial present in soy flour giving [illegible] ion procedures in which the [illegible] ification by bioassay The major steps of the isolation procedure are shown in TABLE 3 Two active barium salts were separated from the final barium precipitate One was in [illegible]

crystallized from hot water, lost a significant proportion of its weight on ashing, and contained about 17 per cent molybdenum Although the purification procedure was not designed to yield inorganic material specifically, nevertheless the isolated substances were molybdate or molybdate complex salts

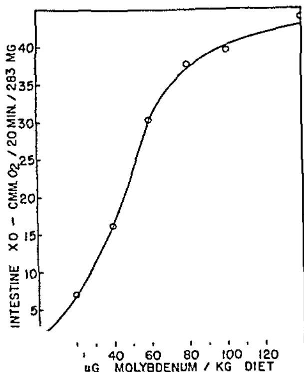
The identification of these substances as molybdates was aided by the concurrent studies of De Renzo *et al* [25] Using the same assay procedure they found that the biological activity was retained in a liver residue ash, and they identified molybdenum as the active component of the ash by testing each of its constituents

The biological activity of sodium molybdate is shown in FIGURE 4 Saturation levels of intestinal xanthine oxidase (above 30) were obtained with diets containing less than 0 1 mgm molybdenum per kgm of diet Higher concentrations of dietary molybdenum gave intestinal XO values averaging between [illegible] molybdenum [illegible]

lower level [illegible]

[illegible] nent of the active material [illegible] ll of the biological [illegible] ybdenum content [illegible] was the case

(1) The activity of the isolated material or of a large number of impure frac



...um molybdate showing the response of intestinal xanthine oxidase to in... the diet

re sodium molybdate when tested on the basis
...ntents
the biological activity of the isolated ma-
...Hence no essential or synergistic organic
...ybdenum during the isolation pro-

...lated material by extracting it as
...moved the biological activity with

... isolated material as an insoluble
... the biological activity
... than enough molybdenum

...e oxidase factor is the trace
...o be required by some plants
...n of a role for molybdenum
...um with an enzyme

TABLE 3

ISOLATION PROCEDURE FOR XOF

(1)	Soy flour reflux with 10 vol of 1N HCl and filter
(2)	Filtrate adsorb activity on charcoal and eluate with ammonia
(3)	Eluate acidify adsorb on alumina column and elute fractionally with 1N ammonia
(4)	[illegible] Wash with 0 2 N HCl
(5)	[illegible]
(6)	[illegible]
(7)	[illegible]

and (4) some dark green, leafy vegetables Foods which were essentially inactive included (1) muscle meats and fish, and (2) fruits and berries The substance present in liver residue and foods, which was responsible for this response could not be identified with any factor known to be required in animal nutrition Nor could it be identified with the antithyrotoxic factor present in liver residue [24]

Isolation and identification of XOF The material present in soy flour giving this response was isolated by a series of fractionation procedures in which the active material was followed throughout its purification by bioassay The major steps of the isolation procedure are shown in TABLE 3 Two active barium salts were separated from the final barium precipitate One was insoluble in water, lost little weight on ashing, and contained 45 per cent barium and 24 per cent molybdenum Spectrographically, it contained only Ba and Mo in amounts greater than 0 1 per cent The other barium salt could be

the isolated substances were molybdate or molybdate complex salts

The identification of these substances as molybdates was aided by the concurrent studies of De Renzo *et al* [25] Using the same assay procedure, they found that the biological activity was retained in a liver residue ash, and they identified molybdenum as the active component of the ash by testing each of its constituents

The biological activity of sodium molybdate is shown in FIGURE 4 Saturation levels of intestinal xanthine oxidase (above 30) were obtained with diets containing less than 0 1 mgm molybdenum per kgm of diet Higher concentrations of dietary molybdenum gave intestinal XO values averaging between 30 and 45, but they were not consistently proportional to the molybdenum content of the diet Hence values above 30 were considered to be saturation levels of intestinal XO, and assays falling within this range were repeated at a lower level

[illegible] established molybdenum as a major component of the active material

(1) The activity of the isolated material or of a [illegible]

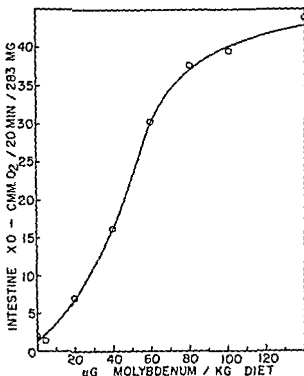


FIGURE 4. Assay curve with sodium molybdate showing the response of intestinal xanthine oxidase to increasing amounts of molybdenum in the diet

tions was identical with that of pure sodium molybdate when tested on the basis of their respective molybdenum contents

(2) The molybdenum content and the biological activity of the isolated material were not decreased by ashing. Hence no essential or synergistic organic component was carried along with the molybdenum during the isolation procedure

(3) A removal of molybdenum from the isolated material by extracting it as an ether soluble thiocyanate complex also removed the biological activity with the molybdenum

(4) Precipitation of molybdenum from the isolated material as an insoluble complex with α-benzoinoxime also precipitated the biological activity

(5) Liver residue and soy flour contained more than enough molybdenum to account for their biological activities.

These results leave no doubt that the xanthine oxidase factor is the trace element molybdenum. Molybdenum is known to be required by some plants and microorganisms but this is the first demonstration of a role for molybdenum in animal nutrition and is the first association of molybdenum with an enzyme

It appears to be a part of the enzyme molecule, since purified preparations of xanthine oxidase contain molybdenum This was demonstrated previously[16] by the assay procedure, and has been confirmed more recently by chemical determinations

Studies of molybdenum deficiency in plants[26, 28] and *Aspergillus niger*[29] have demonstrated a requirement for molybdenum in the reduction of nitrate Xanthine oxidase[30] and aldehyde oxidase[31] are capable of utilizing nitrate or organic nitro compounds[32, 33] as electron acceptors during the oxidation of purine or aldehyde substrates Nitrate is thereby reduced to nitrite,[30] and organic nitro compounds are reduced to hydroxylamino derivatives[33] It seems probable, therefore that the reduction of nitrates by plants molds, *etc* is effected by a molybdenum containing enzyme similar to but not necessarily identical with xanthine oxidase

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STUDIES ON THE NATURE OF THE XANTHINE OXIDASE FACTOR

By Edward C De Renzo

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I should like to extend my thanks to Doctor Schwarz and to Doctor Wester feld for the opportunity of presenting a brief summary of some of our finding on the nature of the xanthine oxidase factor I should like to extend speci thanks to Doctor Westerfeld for his gracious acknowledgment of our contr bution.

When we first became interested in this dietary factor, a study of the literatur revealed that the Syracuse workers were practically alone in thinking about substance other than adequate protein or riboflavin which was concerned wit maintaining tissue xanthine oxidase values In order to satisfy ourselves tha such a factor actually existed, we attempted to reproduce the experiments o [illegible]

response in the intestinal xanthine oxidase value to varying amounts of dietar liver residue and, in addition, rats receiving the control diet alone showed rela tively high levels of this enzyme After a brief talk with Doctors Wester feld and Richert, our problems with the assay were satisfactorily unraveled

TABLE 1 shows the basal diet[1] used in these studies As reported by th Syracuse workers, if weanling rats are given this diet for two weeks, analysis o the small intestine for xanthine oxidase by the manometric technique[2] wi yield a value of about 5 c mm oxygen uptake/20 min /250 mgm intestine Supplementation of this diet with 10 per cent liver residue produces a five- t sevenfold increase in the xanthine oxidase value TABLE 2 shows the respons of rat intestinal xanthine oxidase to varying amounts of liver residue in the die thus confirming the work already reported[3] It should be mentioned how ever that we sometimes experienced difficulty in obtaining xanthine oxidas values proportional to the lower amounts (*i e*, 2 5 per cent) of a liver residu supplement Nevertheless there was never any doubt about the enhance ment obtained with a high level (*i.e.*, 10 per cent) of liver residue and isolatio studies were therefore undertaken

We chose liver residue as our source material since the factor was present i abundant amounts in this material Early in the work it was found that ac tive extracts of xanthine oxidase factor could be obtained by simply auto claving liver residue with water The factor in the extract as well as in th crude liver residue was remarkably stable to acid or alkaline treatment and when the activity was not destroyed by boiling with concentrated sulfuri acid our suspicions as to the nature of the factor were aroused We therefor ashed the active extracts and liver residue, and when the ashed materials wer fed no diminution of activity was encountered These data are shown i TABLE 3

The capable assistance of Mr E Kale and Mr P G Heytler in the performance of these experiment gratefully acknowledged

TABLE 1

BASAL DIET USED IN STUDIES ON XOF

Cerelose	65
Purified casein (Labco)	24
Crisco	4
Cod liver oil	1
Wesson oil	2
Salts (Phillips and Hart[4])	4
Vitamins/kgm diet Riboflavin, 4 mgm Thiamin HCl 4 mgm Pyridoxine HCl, 4 mgm Ca Pantothenate, 10 mgm Nicotinamide, 25 mgm Choline chloride, 1000 mgm	

TABLE 2

RESPONSE OF RAT INTESTINAL XANTHINE OXIDASE VALUES TO LIVER RESIDUE IN THE DIET

Amount of liver residue added to basal diet (per cent)	Av X O value c mm O_2 uptake/20 min /250 mgm intestine
None	5.0
2	10.2
5	22.0
10	29.8

TABLE 3

EFFECT OF LIVER RESIDUE FRACTIONS ON RAT INTESTINAL XANTHINE OXIDASE VALUES

Supplement added to basal diet	Av X O value c mm O_2 uptake/20 min /250 mgm intestine
None	4.4
10% liver residue (LR)	25.6
Liver residue water extract (LRE)	32
Ash of LR	28.2
Ash of LRE	26.9

A spectrographic analysis (TABLE 4) of the ash of the active extract revealed the presence of many elements. The activity of the ash was then found to be replaceable by Hoagland's A–Z solution (50 ml /kgm diet), an inorganic solution containing trace elements and used in studies of plant nutrition.[3] Single salts were then fed in varying amounts in an attempt to ascertain the active substance. On the basis of the elemental composition of the ash and Hoagland's solution and the salt mixture of the basal diet, our studies were restricted to relatively few salts. To our surprise, molybdenum salts were found to be responsible for this effect. These data are shown in TABLE 5. No other salts studied exhibited this effect, and the activity of the ash of active extracts or liver residue could be totally accounted for on the basis of its molybdenum content. Furthermore, and also to our surprise, when an ash of the basal diet was assayed spectrographically, molybdenum was not detected (TABLE 6).

We have attempted to study the effects of molybdenum on milk as well as

TABLE 4

SPECTROGRAPHIC ANALYSIS OF LIVER RESIDUE ASH

Element detected	Approximate per cent (unless Stated as parts per million ppm.)
Al	0 3
Sb	Trace
Ba	0 1
B	1
Ca	1
Cr	30 ppm.
Co	0 1 ppm.
Cu	0 1
Fe	1
Pb	0 3
Mg	10
Mn	30 ppm
Mo	0 3
Ni	30 ppm.
P	3
K	3
Si	10
Ag	30 ppm.
Na	30
Sn	0 03
Tl	10 ppm.
V	0 03
Zn	0 1

TABLE 5

EFFECT OF CERTAIN SALTS ON RAT INTESTINAL XANTHINE OXIDASE VALUES

Supplement added, kgm diet	Av X O value c mm O_2 uptake/23 min /250 mgm intestine
None	7 1
50 ml. Hoagland's solution	21 4
$AlCl_3 \cdot 6H_2O$ 200 mgm	5 3
$CoCl_2 \cdot 6H_2O$ 200 mgm	1 1
$Cr(NO_3)_3 \cdot 9H_2O$ 200 mgm	3 7
$SnCl_4 \cdot 5H_2O$ 200 mgm	3 4
$SrCl_2 \cdot 6H_2O$ 200 mgm	3 7
H_3PO_4 100 mgm	8 6
NaBr 25 mgm	4 9
$K_2Cr_2O_7$ 25 mgm	4 8
$Ti(OH)_4$ 25 mgm	6 7
$Nd(NO_3)_3 \cdot 6H_2O$ 10 to 100 mgm	9 5
$BaCl_2$ 10 to 50 mgm	3 7
$Na_2MoO_4 \cdot 2H_2O$ 0 05 to 100 mgm	27 3

TABLE 6

ELEMENTS DETECTED IN THE BASAL DIET ASH SHOWING THE ABSENCE OF MOLYBDENUM

Al	Ni
Ba	P
Ca	K
Cr	Si
Cu	Ag
Fe	Na
Pb	Sn
Mg	Zn
Mn	

intestinal xanthine oxidase *in vitro* Varying concentrations of molybdate were added to dialyzed or undialyzed active enzyme preparations and enzyme preparations from rats receiving the basal diet (*i e*, deficient in molybdenum) No enhancement of activity has yet been observed under these conditions with any of these enzyme preparations

In preliminary experiments, we have found that the feeding of sodium tung

nal xanthine oxidase preparations has been observed *in vitro*

We have also demonstrated an *in vivo* stimulatory effect of molybdenum on mouse intestinal xanthine oxidase and therefore feel that the participitation of Mo in animal nutrition and metabolism has been firmly established Further attempts are now in progress to demonstrate the precise role of molybdenum on the activity of xanthine oxidase

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THE EFFECTS OF LIVER DISEASE ON CERTAIN ASPECTS OF TOCOPHEROL METABOLISM IN MAN*

By Gerald Klatskin

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The purpose of the present investigation was to characterize the effects of liver disease on certain aspects of tocopherol metabolism in man. Our interest in this problem was stimulated by the observation that chronic biliary fistula leads to vitamin E deficiency in experimental animals,[1, 2] an effect thought to be the consequence of impaired tocopherol absorption. Although clinical evidence of vitamin E deficiency has not been recognized in man, a similar defect in absorption has been postulated to account for the low levels of tocopherol found in liver disease[3, 4]. The subnormal rise in the plasma level following the administration of tocopherol in such cases[3, 4] would appear to lend support to this hypothesis. It is obvious, however, that the plasma level reflects not only the rate of tocopherol absorption in the intestine, but also its rate of storage and utilization in the tissues. Unfortunately, little is known about these aspects of tocopherol metabolism in man, so that the significance of low plasma levels has remained obscure. However, recent evidence implicating tocopherol deficiency in the pathogenesis of experimental dietary hepatic necrosis[5, 6, 7] has served to emphasize the possible importance of this problem. The following studies were undertaken, therefore, with the hope of shedding further light on the interrelationships between hepatic dysfunction and the absorption, utilization, and storage of tocopherol in liver disease.

Results

Plasma tocopherol. Analyses of plasma confirmed previous reports[4] that the average tocopherol level in liver disease is significantly lower than in normal healthy adults (FIGURE 1). It was found, however, that the individual values varied widely, often falling within the normal range, and that the average did not differ significantly from that of a group of randomly selected hospitalized convalescent controls. Moreover, there was no correlation between the concentration of tocopherol and the depth of jaundice or the degree of hepatic dysfunction as judged from the serum levels of bilirubin, cholesterol, and albumin (FIGURE 2).

These observations suggested that the decrease in plasma tocopherol in liver disease was related to some extrahepatic factor, such as impaired absorption, rather than to a metabolic defect in the liver. However, a depletion of the tissues and plasma due to a dietary deficiency of tocopherol had to be excluded, since many of the subjects in both the liver disease and convalescent control groups had subsisted on grossly deficient diets for long periods.

Saturation tests. If the fall in plasma tocopherol were indeed related to such a deficiency, repletion of the tissues might be expected to effect a sustained rise in the plasma tocopherol level. For that reason, the effects of large amounts

* This investigation was supported by a research grant A-4 (C7) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

intestinal xanthine oxidase *in vitro* Varying concentrations of molybdate were added to dialyzed or undialyzed active enzyme preparations and enzyme preparations from rats receiving the basal diet (*i e*, deficient in molybdenum) No enhancement of activity has yet been observed under these conditions with any of these enzyme preparations

In preliminary experiments, we have found that the feeding of sodium tungstate to rats receiving minimal amounts of molybdenum inhibits the stimulation of intestinal xanthine oxidase This appears to be an interesting sort of ion antagonism. However, no inhibitory effect of tungstate on milk or intestinal xanthine oxidase preparations has been observed *in vitro*

We have also demonstrated an *in vivo* stimulatory effect of molybdenum on mouse intestinal xanthine oxidase and therefore feel that the participitation of Mo in animal nutrition and metabolism has been firmly established. Further attempts are now in progress to demonstrate the precise role of molybdenum on the activity of xanthine oxidase

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The purpose of the present investigation was to characterize the effects of liver disease on certain aspects of tocopherol metabolism in man. Our interest in this problem was stimulated by the observation that chronic biliary fistula leads to vitamin E deficiency in experimental animals,[1,2] an effect thought to be the consequence of impaired tocopherol absorption. Although clinical evidence of vitamin E deficiency has not been recognized in man, a similar defect in absorption has been postulated to account for the low levels of plasma tocopherol found in liver disease.[3,4] The subnormal rise in the plasma level following the administration of tocopherol in such cases[3,4] would appear to lend support to this hypothesis. It is obvious, however, that the plasma level reflects not only the rate of tocopherol absorption in the intestine, but also its ate of storage and utilization in the tissues. Unfortunately, little is known bout these aspects of tocopherol metabolism in man, so that the significance low plasma levels has remained obscure. However, recent evidence implicat tocopherol deficiency in the pathogenesis of experimental dietary hepatic crosis[5,6,7] has served to emphasize the possible importance of this problem. following studies were undertaken, therefore, with the hope of shedding further light on the interrelationships between hepatic dysfunction and the absorption, utilization, and storage of tocopherol in liver disease.

Results

Plasma tocopherol. Analyses of plasma confirmed previous reports[4] that the average tocopherol level in liver disease is significantly lower than in normal healthy adults (FIGURE 1). It was found, however, that the individual values varied widely, often falling within the normal range, and that the average did not differ significantly from that of a group of randomly selected hospitalized convalescent controls. Moreover, there was no correlation between the concentration of tocopherol and the depth of jaundice or the degree of hepatic dysfunction as judged from the serum levels of bilirubin, cholesterol, and albumin (FIGURE 2).

These observations suggested that the decrease in plasma tocopherol in liver disease was related to some extrahepatic factor such as impaired absorption, rather than to a metabolic defect in the liver. However, a depletion of the tissues and plasma due to a dietary deficiency of tocopherol had to be excluded, since many of the subjects in both the liver disease and convalescent control groups had subsisted on grossly deficient diets for long periods.

Saturation tests. If the fall in plasma tocopherol were indeed related to such a deficiency, repletion of the tissues might be expected to effect a sustained rise in the plasma tocopherol level. For that reason, the effects of large amounts

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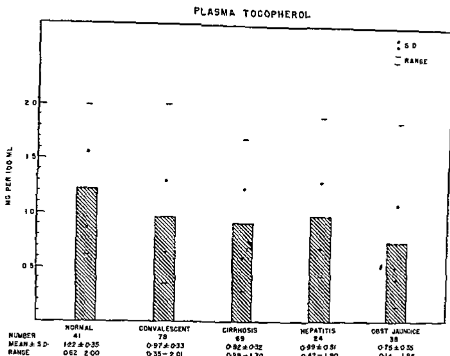


FIGURE 1

of orally administered tocopherol were investigated (The 4 gm dose of acetate employed approximated the estimated total body content of tocopherol)[5]

It can be seen in A and B of FIGURE 3 that, with the exception of the obstructive jaundice group, the subjects with liver disease showed as great a rise in plasma concentration during tocopherol administration as the normals, suggesting that absorption from the intestine was not impaired However, as is evident in D of FIGURE 3, the rise was sustained in the cirrhotic group only In the latter, moreover, the absolute increase in concentration over the control level actually exceeded that in the normals (C of FIGURE 3)

These data suggested that dietary deficiency was at least one of the factors responsible for the low plasma tocopherol levels in cirrhosis If it played any role in the other types of liver disease investigated, it was obscured by other more important factors

Tolerance tests To further evaluate the factor of intestinal absorption, tolerance tests based on plasma curves following the oral administration of tocopherol were carried out These confirmed previous reports[4] that the curves in liver disease tended to be lower and flatter than normal (A of FIGURE 4) Further analysis, however, revealed that while the peak values attained were low (B of FIGURE 4), the increases above the initial fasting level (C of FIGURE 4), and the mean rises in concentration estimated from the areas under the curves (D of FIGURE 4), were within normal limits, except in the case of obstructive jaundice

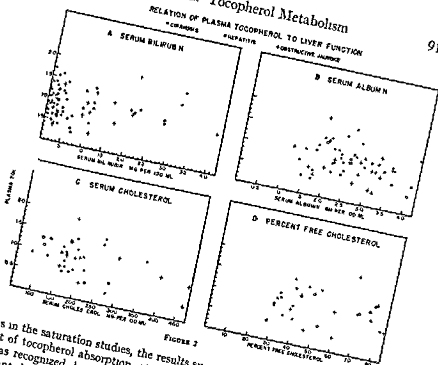


FIGURE 2

As in the saturation studies, the results suggested that there was no impairment of tocopherol absorption, at least in the cirrhotis and hepatitis groups. It was recognized, however, that estimates based on plasma curves did not warrant definite conclusions regarding intestinal absorption, unless paralleled by measurements of tocopherol excretion. For that reason, it was necessary to carry out a number of balance experiments. Since preliminary investigation revealed that little or no tocopherol was excreted in the urine, these were based on analyses of feces.[9]

Fecal tocopherol. To our surprise, it was found that subjects with liver disease regularly excreted a smaller fraction of ingested tocopherol than normals, both on their usual diets (A and B of FIGURE 5) and following the ingestion of a large tocopheryl acetate supplement (C of FIGURE 5). Several possible explanations for the low fecal tocopherols in liver disease were considered.

(1) A larger fraction of ingested tocopherol was absorbed, (2) A fraction of the ingested tocopherol was excreted as the acetate and, therefore, escaped detection in the Emmerie Engel reaction, which served as the basis for the analytical procedure employed,[10]

(3) There was interference with some as yet unrecognized normal mechanism for the re-excretion of tocopherol into the intestine,

(4) Synthesis of tocopherol occurred in the intestinal tract of the normals and was diminished in the subjects with liver disease,

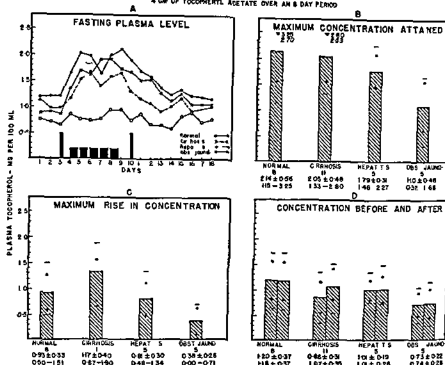


FIGURE 3

(5) There was an increased rate of tocopherol breakdown in the intestinal tract

(*1*) *Increased absorption* If the subjects with liver disease did in fact absorb a larger fraction of ingested tocopherol, it would have to be assumed that the relatively low or normal plasma curves observed during tocopherol absorption (FIGURE 4) were the consequence either of an increased rate of utilization or of a more rapid diversion of tocopherol to the tissues Under these conditions, loading of the plasma and tissues with large amounts of tocopherol might be expected to result in a relative decrease in the rate of utilization and/or transfer [illegible]

[illegible]

curves can be used as an index, did it alter the rate of [illegible] Unfortunately, more direct means for measuring utilization could not be devised Nevertheless, it was concluded tentatively that the low fecal tocopherol levels in liver disease were not due to an increase in tocopherol absorption

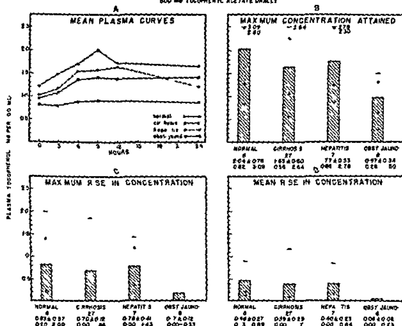


FIGURE 4

(2) *Tocopheryl esters* When the feces were saponified to free tocopherol

organisms (B of FIGURE 7) indicating that the esterases responsible for this

for its reduction in liver disease

FECAL EXCRETION OF TOCOPHEROL

Figure 5

TOCOPHEROL TOLERANCE TESTS BEFORE AND AFTER "SATURATION"

Figure 6

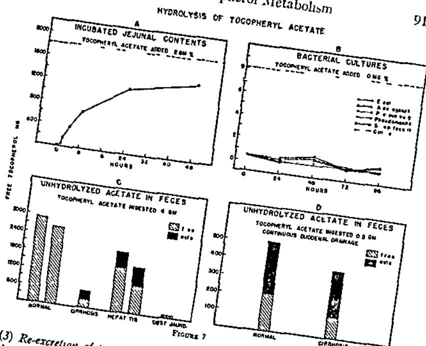


FIGURE 7

(3) Re-excretion of tocopherol The possibility that there was interference with some normal mechanism for the re-excretion of tocopherol into the intestinal tract, which led to a reduction in fecal tocopherol, was suggested by the observation that the amount excreted in liver disease remained significantly above the baseline level for at least 9 days following an oral test dose of tocopherol (D of FIGURE 5) Partial confirmation of such a mechanism was obtained from analyses of T tube bile in subjects with obstructive jaundice These revealed concentrations of tocopherol comparable to those in plasma (A of FIGURE 8) However, it was not possible to demonstrate any interference with this enterohepatic circulation of tocopherol in liver disease Thus, analyses of duodenal contents aspirated for a period of 24 hours following instillation of tocopherol distal to an occlusive balloon in the jejunum failed to reveal any difference in the amount recovered in normal and cirrhotic subjects (C of FIGURE 8) Moreover, the fact that the amount recovered was small and the observation that the concentration in T tube bile did not increase following the ingestion of tocopherol (B in FIGURE 8) suggested that the bile contributed very little to the total fecal tocopherol in the previously described balance experiments

It was concluded, therefore that interference with the enterohepatic circulation of tocopherol was not responsible for the low fecal levels found in liver disease The possibility of a defect in its re-excretion, however, through the intestinal mucosa could not be excluded, although it appeared unlikely

BILIARY EXCRETION OF TOCOPHEROL

A

RELATION OF BILIARY TO PLASMA CONCENTRATION OF TOCOPHEROL

○α tocopherol ●tocopherol B

BILE TOCOPHEROL MG PER 100 ML

0.5 1.0 1.5 2.0

PLASMA TOCOPHEROL MG PER 100 ML

0.5 1.0 1.5

B

CONCENTRATION IN T TUBE BILE DURING TOCOPHEROL ADMINISTRATION

tocopheryl acetate 4 gm.

0.5 1.0 1.5 2.0

C.P.

J.M.

DAYS

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

C

TOCOPHEROL RECOVERED IN DUODENUM

ASPIRATION FOR 24 HOURS FOLLOWING INSTILLATION OF 500 MG TOCOPHERYL ACETATE INTO THE JEJUNUM DISTAL TO AN OCCLUSIVE BALLOON

free ester

MILLIGRAMS

10 20 30 40

NORMAL CIRRHOSIS

FIGURE 8

STABILITY OF TOCOPHEROL DURING INCUBATION AT 37°C

A

FECAL EMULSION

N.K. CIRRHOTIC

FOLLOWING INGESTION OF TOCOPHERYL ACETATE

0 24

HOURS

B

ASPIRATED JEJUNAL CONTENTS

1400 1500 1600 1700

N.R. INTESTINAL OBSTRUCTION

TOCOPHERYL ACETATE ADDED IN VITRO

0 8 16 24 32 40 48

HOURS

C

BROTH CULTURES

E. coli

A. aerogenes

Pseudomon.

Control

ADDED TOCOPHEROL

24 48 72 96

HOURS

D

BROTH CULTURES

A. aerogenes

St. fecalis

Proteus v.

Control

Pseudomon.

E. coli

2 4 6 8

ADDED TOCOPHEROL

0 24 48 72 96

HOURS

FIGURE 9

(4) Tocopherol synthesis The possibility of tocopherol synthesis in the intestinal tract was investigated, although there was no previous evidence to indicate that it was a factor in determining the level of tocopherol in the feces. Attempts to demonstrate such synthesis in cultures of a broad spectrum of aerobic and anaerobic enteric organisms grown in a variety of enriched media, or in incubated emulsions of feces and aspirated intestinal contents, were uniformly unsuccessful.

(5) Tocopherol "destruction" Largely by exclusion, an increased rate of destruction in the intestinal tract appeared to be the most likely explanation for the low fecal tocopherol in liver disease. It was not possible, however, to demonstrate this by direct experiment. Thus, there was no loss of tocopherol during its prolonged incubation in fecal emulsions (A of FIGURE 9), aspirated jejunal contents (B of FIGURE 9), or cultures of enteric organisms (C and D of FIGURE 9). Nevertheless, it must be emphasized that these experimental conditions by no means duplicated those in the intestinal tract so that differences in tocopherol destruction were not excluded. The recent observations of Rosenkrantz and his associates[11] are highly significant in this connection. These investigators found that an appreciable fraction of the tocopherol in normal feces was excreted as the quinone, a form not detectable by the Emmerie Engel reaction. It is conceivable, therefore, that a more rapid oxidation of tocopherol to tocopherylquinone in the intestinal tract was responsible for the low fecal values obtained by the Emmerie-Engel reaction in the present experiments. Further studies along these lines are contemplated.

Summary

In summary, then, previous reports of low plasma tocopherol levels in liver disease have been confirmed. The evidence presented, however, suggests that these low levels are not the result of impaired absorption but are, rather, the consequence of an increased rate of tocopherol breakdown in the intestinal tract. Moreover, in some instances dietary deficiency appears to be a contributory factor.

Acknowledgment

The author is indebted to Doctor David W Molander, Doctor Willard A Krehl, Doctor Daniel Marshall and Miss Rita Pacelli for their assistance in carrying out these studies.

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PHOSPHOLIPIDE SYNTHESIS IN LIVER METABOLISM*

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Phospholipide synthesis is a primary function of all cells in the body. They make up part of the cell structure, being found in both the cytoplasma and nucleus. Mitochondria and large granules are very rich in phospholipides[1]. These intracellular structures contain enzymes which can oxidize fatty acids[2] and other metabolites. The liver is the main source of phospholipides in plasma[3]. Plasma phospholipides show a direct reflection of turnover in the liver[4,5]. The choline-containing phospholipides are probably an integral part of enzyme systems necessary for the oxidation of fats and fatty acids. The lipotropic effect of lecithins appears to act on the metabolism of fatty acids in the liver, rather than by enhancing their mobilization in the form of plasma phospholipides. The composition of liver lipides can be altered by the dietary intake of proteins[6,7]. There is usually a decrease in the level of total phospholipides in the liver of animals maintained on a low protein diet. The decrease is especially marked in the lecithin fraction with a consequent lower ratio of the choline-containing to the total phospholipides. Various studies comparing the rate of incorporation of P^{32} into the liver lipides of animals on various experimental diets have been made[6,7,8].

The phospholipide turnover in the plasma of normal individuals varies widely but is rather constant in the same individual over a period of many months[9]. The effect of a low protein diet (7.6 per cent) or a normal diet for 9 days seems to have very little change in the rate of synthesis of the plasma phospholipide in man[10]. The phospholipides of the plasma are primarily choline-containing, but in the liver, both choline and noncholine-containing are abundant[11,12]. Attempts have been made to study the effects of dietary requirements on the synthesis of these two fractions in the liver[13]. The synthesis of the choline containing fraction was practically the same in the liver of animals maintained on a low protein diet (5 per cent) as in those on a stock diet or a 25 per cent casein diet. The only instance in which a marked decrease in synthesis of the choline containing fraction were those of experiment, in which guanidoacetic acid or diethanolamine was supplemented[14]. Diethanolamine apparently acts as a metabolic antagonist of ethanolamine for the formation of lecithin as well as the natural cephalin[15]. The rate of lecithin synthesis by the liver of the rat is apparently related to the activity of choline oxidase, or to the role of natural inhibitors such as fatty acids[16]. This diminished choline oxidase activity permits an adequate rate of synthesis of phospholipides containing choline. It is impossible to produce a "fatty" liver by dietary means in the liver of the guinea pig, since this enzyme is not present in The liver cell is more efficient with the lecithin molecule when the "fatty" even though a decrease in the total phosph

* This investigation was supp... Fellowship of the ... Society

TABLE 1

*PHOSPHOLIPIDE SYNTHESIS IN THE RAT AND GUINEA PIG ON STOCK DIET**

Animal	No	Body weight gm	Phospholip de P			Phospho-l pide-P/ Nitrogen	Relative radio-activity per gm of fat free t ssue
			mgm	*Relative specific act vity*			
				overall	per gm of fat free tissue		
Rat	8	278	17 4	0 386	0 040	0 054	0 68
Guinea pig	6	989	33 5	0 258	0 009	0 042	0 30

* Animals k lled 6 hours after the nject on of P^{32}

A comparison of phospholipide synthesis in the guinea pig and rat has been made in an attempt to determine the role of choline oxidase in lipide phosphorylation (TABLE 1)[18] In rats and guinea pigs maintained on stock diets the phospholipide P/N was unaltered The relative specific activities of the phospholipides of the guinea pig are only slightly decreased from those of the rat This difference becomes less when the relative radioactivity values of the guinea pig liver lipides are calculated for 1 gm of fat free tissue, which should represent the activity of a definite weight of tissue in synthesis phospholipides We may conclude that the synthesis of phospholipides by the

heavy metals, has long been known Infections in different parts of the body, as well as parasitization of the liver cells themselves, alter liver functions[19] *The phospholipide synthesis in the plasma of untreated cirrhotic patients is* very similar to that found in normal persons[20] The phospholipide turnover in the liver of rats in experimental *cirrhosis has been produced* by carbon tetrachloride and has been found to be unaltered[8, 21] The phospholipide

is constant when expressed as per gram of fat free tissue Attempts to compare the rate of incorporation of P^{32} into the liver phospholipides and nucleoproteins of rats have been made (TABLE 2)[24] Male rats (100–110 gms) were maintained 4–8 weeks on diets containing 25 per cent, 5 per cent Casein with respectively a low (5 per cent) or high (32 per cent) fat content The rats ... ected with P^{32} and after 6 hours the inorganic P, lipides and nucleo-

activities of the phospholipide ...

TABLE 2

AVERAGE VALUES OF PHOSPHOLIPIDE AND "NUCLEOPROTEIN" TURNOVER IN THE LIVER OF RATS ON VARIOUS DIETS

Diet: Protein %	Diet: Fat %	Number of animals	Body weight gm	Total lipides gm	Phospholipide P: Relative specific activity: overall	Phospholipide P: Relative specific activity: per gm of fat free tissue	Nucleoprotein P: Relative specific activity	Phospholipide P/Nitrogen	R S A Phospholipide-P*/R S A Nucleoprotein P
5	5	16	114	1 47	0 466	0 087	0 254	0 037	1 840
25	5	10	230	0 54	0 390	0 044	0 178	0 037	2 190
5	32	11	130	2 46	1 045	0 167	0 389	0 050	2 690

* R S A. = Relative specific activity All animals maintained on the diet 8 weeks

TABLE 3

AVERAGE VALUES FOR PHOSPHOLIPIDE AND "NUCLEOPROTEIN" TURNOVER IN THE LIVER OF RATS MAINTAINED ON A LOW PROTEIN LOW FAT DIET*

Days on diet	Number of animals	Body weight gm	Total lipides gm	Phospholipide-P: Relative specific activity: overall	Phospholipide-P: Relative specific activity: per gm of fat free tissue	Nucleoprotein P: Relative specific activity	Phospholipide P/nitrogen	R S A Phospholipide-P†/R S A nucleoprotein P
64	11	101	2 33	0 210	0 035	0 084	0 037	2 508
96	4	148	4 07	0 179	0 030	0 071	0 040	2 663
114	8	126	3 21	0 200	0 032	0 093	0 039	2 175
164	12	169	3 09	0 177	0 025	0 102	0 029	1 767
185	6	152	2 36	0 259	0 046	0 137	0 037	2 040
206	14	142	2 99	0 181	0 028	0 083	0 033	2 211

* 5% protein and 5% fat
† R S A = relative specific activity

intake or by the amount of fat in the liver Campbell and Kosterlitz[23] have shown that the phospholipide P content of a unit of liver cell is determined mainly by the dietary protein intake, a little by the fat intake, and not at all by the choline content of the diet The ratio of phospholipide P/protein N, is constant over a wide range of dietary protein intake [25]

Attempts to compare the rate of phospholipide and nucleoprotein synthesis in the production of experimental cirrhosis have been made (TABLE 3) In rats maintained on a 5 per cent Casein diet for 4, 8, 13, 16, and 28 weeks, the ratios of the synthesis of phospholipides to nucleoprotein and phospholipide P/N were constant and were not related to the degree of cirrhosis or fibrosis by histological evidence There appears to be a constant relation of the synthesis of phospholipide to that of the nuceloprotein, whether or not the liver is "fatty," fibrosed, or normal [24]

This constant rate of synthesis of liver phospholipide appears changed very

TABLE 4

EFFECT OF THIOUREA ON THE PHOSPHOLIPIDE TURNOVER IN THE LIVER OF RATS

Wks on exp	Substance given	No of rats	Specific activity				
			Inorganic P	Lipide P		Lipide P × 100 / inorganic P	
					% change		% change
3	None	4	152	101		67	
	Thiouracil	4	157	79	−22	50	−25
	Thiouracil*	4	164	78	−23	47	−30
5	None	4	173	115		67	
	Thiouracil	4	187	96	−16	51	−24
	Thiouracil†	4	213	110	−4	52	−22

Rats fed a high protein diet (thiouracil 0 3%) then a low protein (thiouracil 0 6%) last week of experimer
* Last day 100 mgm thiouracil by stomach tube
† Last day 100 mgm thiourea by stomach tube

TABLE 5

THE EFFECT OF THYROID GLAND ON THE PHOSPHOLIPIDE TURNOVER IN THE PLASMA OF MA

Patient	Exp conditions	Relative specific activities of plasma phospholipide		
		24 hours	48 hours	72 hours
R B	Thyrotoxicosis	0 731	1 588	1 85
	After surgery	0 292	0 805	1 03

little by dietary intake, but is influenced by metabolic requirements of the body *The thyroid gland plays a definite role in this metabolism*[26] In experimental animals thyroxine increases the rate of synthesis both in plasma and liver[27] Thiouracil and thiourea diminish the phospholipide turnover[27, 28] As shown in TABLE 4, a significant decrease in the animals receiving the drugs [illegible] apparent The plasma phospholipide turnover in a patient with clinical [illegible] showed a decreased rate of syn [illegible]

Lipide phosphorylation is greatest in conditions in which a single dose of lipotropic agents are administered in the presence of a "fatty" liver The effect of a dose of choline is chiefly due to an increased rate of formation of lecithins[29] The stimulating effect of various incomplete methylated ethanol amine derivatives was greatest in the choline containing fraction in rats maintained on a low protein diet[30] In cirrhotic patients with fatty infiltration of the liver by biopsy a significant increase in the rate of phospholipide synthesis is usually demonstrable after a single dose of choline or methionine[31]

The stimulating action of lipide phosphorylation in the liver has a low degree of structural specificity than other biological actions exerted by the same substance[32] Thus there are substances which, without being lipotropic, increase the turnover rate of liver phospholipides, *e g*, *cystine*,[33] *ethanolamine*,[34, 15] and diethanolamine[15] In animals maintained on a low protein diet, the stimulat

Table 6

The Effect of Cortisone on the Ratio of Phospholipide and Nucleoprotein Turnover in the Rabbit*

Tissue	Number of animals	Material given	Relative specific activity of phospholipide P / Relative specific activity of nucleoprotein P
Liver	10	—	1 32
	13	Cortisone	1 62
Kidney	4	—	1 94
	6	Cortisone	1 05
Lung	4	—	1 00
	7	Cortisone	1 15
Heart	4	—	0 62
	7	Cortisone	0 61
Skeletal muscle	4	—	0 69
	9	Cortisone	0 61

* Cortisone 7 5 mgm I M daily for two months

ing effect of ethanolamine was most pronounced in the noncholine-containing fraction of the liver lipides[30] Diethanolamine presumably acts as a metabolic antagonist of the methyl acceptors in the synthesis of choline with the accumulation of an atypical cephalin[18] This stimulatory effect of lipide phosphorylations by lipotropic agents is somewhat related to the previous diet for it does not occur in rats on a stock diet[36] or in man in adequate protein intakes[9, 10] Many other compounds have to be studied for their stimulating effect of lipide phosphorylations[32] Cortisone administered for two months in the rabbit, failed to change the rate of lipide phosphorylation in the liver (Table 6)[17] Apparently, the rate of synthesis of phospholipide by the liver cell is rather constant when related to a unit of tissue or nucleoprotein and is not influenced by the protein content of the diet fat content of the liver or degree of fibrosis The stimulating effect of various lipotropic agents may represent a replacement of a deficiency of methyl donors or methyl acceptors[12, 13] or merely a mass action effect[15]

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ANTIBIOTICS AND LIVER INJURY*

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Dietary factors determining experimental injury in rats are summarized in TABLE 1

The dietary factors beneficial in the prevention of cirrhosis as the more chronic form of hepatic injury may be identified by one common denominator, *i e by a sufficient supply of lipotropic factors, in particular of choline and its* precursors The benefit seen after administration of Vitamin B_{12} may be due to its choline sparing effect In more recent studies we found hog mucin effec

appears to improve protein utilization

The acute form of experimental dietary hepatic injury, acute massive necrosis of the liver, may be prevented by the sulfur containing amino acids, cystine or methionine, or by vitamin E In addition to these dietary factors, other dietary constituents such as Factor 3 of Schwarz and to some extent fats rich in monoglycerides as well as some emulsifiers (for example, Tween 60) may delay the development of experimental dietary hepatic necrosis

In contrast to cirrhosis it is difficult to reconcile pure deficiency as the possible cause of dietary hepatic necrosis with the interchangeability of substances chemically as different as the sulfur-containing amino acids, cystine or methionine, and the fat soluble vitamin E in the prevention of hepatic necrosis The assumption has been made that the beneficial effect of the sulfur containing amino acids and tocopherol more probably results from an underlying

substances In the case of dietary hepatic necrosis such toxic substances may originate in the intermediary metabolism or under the influence of the intestinal flora particularly in the large intestine

Our first approach was directed toward the elimination—or at least modification—of the intestinal flora as a possible source of factors injurious to the liver It has been shown that aureomycin, when added to the necrogenic experimental basal diet containing yeast as the sole source of protein, had a significantly beneficial effect in the prevention of experimental hepatic necrosis in rats In contrast to vitamin E or the sulfur-containing amino acids, cystine or methionine which as supplements to the basal experimental diet will permanently prevent the production of hepatic necrosis, aureomycin was found to delay as a rule the appearance of necrosis and thus its protective action was only temporary

Sponsored by the Commission on Liver Diseases Armed Forces Epidemiological Board and supported in part by the Office of The Surgeon General Department of the Army

TABLE 1

DIETARY FACTORS IN LIVER INJURY

	Cirrhosis	Necrosis
Protein	Beneficial	Beneficial
Methionine	Beneficial	Beneficial
Cystine	Injurious	*Beneficial*
Choline	Beneficial	*No effect or injurious*
Vitamin E	No effect	Beneficial
Dietary fat	Injurious	*No effect or injurious*
Vitamin B_{12}	Beneficial	No effect

In analyzing this effect of aureomycin, it should be pointed out that intensive studies have revealed no indication of an underlying systemic or focal hepatic infection as the direct cause of experimental dietary acute necrosis of the liver. Thus the choice lies among (1) the supply of a missing antinecrogenic substance, (2) a direct metabolic, and (3) an antimicrobial effect on the intestinal flora. As one possible "contaminant" we have used vitamin B_{12} as a supplement to the basal diet, with or without added aureomycin, and found it without any appreciable effect. Although a direct metabolic effect of aureomycin could not be excluded and it still remains a possibility, we favored interaction between aureomycin and the intestinal flora as the determining factor.

If the effect of aureomycin is mediated by the suppression of the intestinal flora, other antimicrobial agents should also prove to be effective, although not necessarily equal to aureomycin depending mainly on their bacterial "spectrum" and on the ease with which they may produce resistant strains. Thus, it seemed advisable to study the effect of various antimicrobial agents on the production of dietary hepatic necrosis, especially in comparison with the effect of aureomycin.

In one of the experiments we compared aureomycin with polymyxin and streptomycin, and found polymyxin ineffective, streptomycin slightly effective and aureomycin very significantly effective. The delay in the production of massive necrosis by aureomycin becomes particularly evident when the number of surviving animals is charted in relation to days of survival.

In addition to polymyxin, chloramphenicol and bacitracin were without effect on the production of dietary hepatic necrosis in rats. Sulfaguanidine had a slightly protective effective effect, whereas streptomycin, neomycin, and terramycin in increasing order were definitely effective.

Ingested streptomycin is absorbed from the intestinal tract only in traces. Thus its beneficial effect further suggests suppression of the intestinal flora as the mode of action. Observations with penicillin are in good accord with this assumption. Whereas penicillin given by injection was either without any, or of only limited beneficial effect, penicillin given by mouth, especially its poorly soluble organic base salts, exerted very marked protection, not less than that of aureomycin.

With regard to this comparison between aureomycin and oral penicillin, the qualifying remark has to be made that during the course of these investigations,

TABLE 3

Treatment	Total liver fat (%)	Food intake (gm /day)
Aureomycın	25 4 ± 2 0	6 5 ± 0 2
Terramycın	12 2 ± 2 2	6 1 ± 0 2
Methıonıne	8 8 ± 0 7	6 7 ± 0 2
Aureomycın Methıonıne	13 2 ± 2 4	6 6 ± 0 2
Terramycın Methıonıne	7 5 ± 0 7	6 0 ± 0 2
	7 6 ± 0 9	6 0 ± 0 3

TABLE 4

No of rats	Sex	Diet	Av wgt gain	Cırrhosıs
10	M	Y5H	−41 2 ± 11 0	9
10	M	Y5H + A	+16 3 ± 6 5	0
10	F	Y5H	−34 8 ± 6 4	10
10	F	Y5H + A	+19 6 ± 3 6	2

production of fatty lıver and dıetary cırrhosıs ın rats fed a cırrhosıs-producıng ratıon low ın proteın (caseın) and hıgh ın fat In such rats, they exerted a very *pronounced lıpotropıc effect This effect may be noted on Table 3*

Aureomycın had a hıghly protectıve effect on dıetary cırrhosıs and ıts sequelae, such as ascıtes and renal ınjury See Table 4

Sımılar results were obtaıned wıth penıcıllın mıxed wıth the food eıther ın ... Chloro

... med

... t on

hepatıc necrosıs Note Table 6

Durıng the last few years, the growth promotıng effect of antıbıotıcs has been put to a successful practıcal test ın anımal husbandry and ıs generally ...

... of acute

... be ex plaıned by the elımınatıon of bacterıa from the ıntestınal tract whıch may use up protectıve constıtuents of the food ıngested and ın consequence reduce theır supply for the body Such mechanısm may more easıly explaın the effect of antıbıotıcs on the preventıon of cırrhosıs and growth than on the preventıon of massıve necrosıs whıch, wıth the ınterchangeabılıty of cystıne and tocopherol as protectıve factors makes the ınteractıon of toxıc factors perhaps as a result of 'ıntestınal autoıntoxıcatıon,' a reasonable possıbılıty to be strongly consıdered

Sparıng of an essentıal dıetary constıtuent under ınfluence of antıbıotıcs has

TABLE 5

THE EFFECT OF ANTIBIOTICS ON THE DEVELOPMENT OF LIVER CIRRHOSIS IN RATS FED PEANUT MEAL DIET

Group	Supplement (mgm /daily)	Weight (gm)		Cirrhosis		Kidney injury	
		Starting	Final	0	+	0	+
1	—	154 ± 2 7	252 ± 19 1	2	8	4	6
2	Aureomycin 5	154 ± 3 0	310 ± 7 8	8	2	8	2
3	Aureomycin 25	154 ± 2 7	307 ± 6 2	10		10	
4	Terramycin 5	154 ± 2 4	305 ± 6 1	8	2	8	2
5	Terramycin 25	152 ± 2 2	327 ± 4 6	10		10	
6	Penicillin 25	153 ± 1 8	307 ± 3 3	10		10	
7	D benz Pen 5	153 ± 2 1	325 ± 2 7	10		10	
8	D benz Pen 25	153 ± 2 3	320 ± 5 8	10		10	
9	Chloromycetin 5	152 ± 2 0	307 ± 10 0	7	3	9	1
10	Chloromycetin 25	152 ± 1 9	309 ± 5 5	10		10	

(Experimental period 150 days with 10 rats in each group)

TABLE 6

	Weight gain during first four weeks gm
Controls	4 4 ± 1 3
Aureomycin	24 0 ± 1 05
Polymyxin	12 0 ± 1 05
Streptomycin	25 5 ± 0 9

been recently demonstrated by Popper and his associates on the example of choline Two thirds of choline ingested is excreted by a normal person in the urine as trimethylamine or its oxidized form Priming with aureomycin or penicillin will reduce the excretion of trimethylamine after ingestion of choline to very low levels Intravenous injection of choline will not lead to urinary excretion of trimethylamine These observations seem to indicate that the intestinal flora even in a normal person may convert a large proportion of ingested choline into trimethylamine Suppression of the intestinal flora or, at least, of its choline-metabolizing constituents by aureomycin or penicillin will make more choline available for the body By analogy, similar mechanisms may be postulated for other lipotropic food constituents, effective in the prevention of dietary cirrhosis

Such easy solution however is not borne out by more intensive analysis of all participating factors In the first place in collaboration with Popper and his associates, we found that the sparing of dietary choline under the influence

cient to explain the beneficial result which follows prolonged administration of antibiotics

Further, in intensive studies we were unable to demonstrate any permanent change in the composition of the intestinal flora as the result of antibiotic action In these studies carried out in cooperation with Doctor Shockman Doctor Forbes, and Miss Chu temporary suppression of sensitive microorganisms is followed by the return of the original flora characteristic for the diet in question The only remarkable and significant difference is the emergence of resistant strains Thus it is our present assumption that all the metabolic changes such as prevention of hepatic injury or growth promotion must be linked with changed metabolism of the microorganisms comprising the symbiotic intestinal flora There are indications that such changes may be reflected in the metabolism of the host organism as well such as in the urinary composition Our first effort was concentrated on the study of sulfur metabolism with special reference to fecal and urinary sulfur excretion No difference could be found in the S metabolism or in the urinary excretion of gluconic acid in rats fed the necrogenic yeast diet with or without antibiotics The most promising results were obtained through the study of ether soluble acids in the urine In cooperation with Doctor Barness and Doctor Forbes we found that rats fed the necrogenic diet supplemented with aureomycin or penicillin have a low total ESA excretion

[illegible]

isosuccinic acid and hippuric acid Spot 95 is α α-dimethylsuccinic acid Spots 45 and 86 are as yet unidentified α α Dimethylsuccinic acid makes up a greater proportion of the ESA in the aureomycin and penicillin treated animals Spot 86 does not appear in the nonsupplemented animals or in animals receiving vitamin E Spot 45 is of greater intensity in the supplemented than in the nonsupplemented or vitamin E treated animals

The relation of these peculiar metabolic patterns to hepatic necrosis is under further study

Discussion of the Paper

DOCTOR KLAUS SCHWARZ It is a well established fact that dietary necrotic liver degeneration is a deficiency disease that can be prevented by cystine vitamin F and Factor 3 Comparatively speaking it is conjectural and more [illegible] involved in [illegible]

cause of [illegible] intestinal intoxication was held to be the reason of diseases such as hyper thyroidism and pernicious anemia

That antibiotics such as aureomycin have a strong inhibitory influence on

dietary necrotic liver degeneration is nothing unusual or special It has been very clearly demonstrated in various laboratories that almost all vitamin B deficiencies in the rat can be inhibited or even prevented by the supplementation of various antibiotics[1, 2, 3, 4] Their mode of action has not been clarified I am sure, however, that it would be unjustified to infer from such observations that all these different manifestations of avitaminosis are "gastrointestinal intoxications"

References to the Discussion

1 [illegible]

2 [illegible]

3 [illegible]

4 [illegible]

GERMFREE ANIMALS AND LIVER NECROSIS*

By T D Luckey, J A Reyniers, Paul Gyorgy, and M Forbes

LOBUND Institute for Research in the Life Sciences, University of Notre Dame, Notre Dame Ind, and School of Medicine, University of Pennsylvania, Philadelphia, Pa

During the latter part of 1949, discussions between the group working on the etiology of massive hemorrhagic necrosis of the liver at the University of Pennsylvania and the research staff at LOBUND Institute led to preliminary experiments early in 1950 to explore the possibility of using germfree rats in this study It was found that the syndrome was readily produced in conventional rats from the LOBUND strain and that autoclaving the diet[1] did not materially alter its necrogenic character Critical experimentation with germfree rats should determine whether the microbial flora affects the incidence of liver necrosis The results obtained with germfree rats up to the present date are summarized in this report

Methods

Germfree animals were reared and maintained in the Reyniers germfree system[2] The germfree units are provided with locks in which materials such as food and water can be introduced into the cage with steam sterilization Sterile air is obtained via glass fiber filtration The operator cares for the animals through attached rubber gloves The animals are housed in ordinary wire bottom rat cages or in metabolism cages when urine and fecal samples were collected At weekly intervals, bacteriological examinations are made to establish whether the cage is germfree[3]

The diet used was patterned after that of Himsworth[4] The composition is corn starch 79 5 gm, British brewer's yeast, 18 0 gm, salts (USP III), 3 0 gm, peanut oil, 5 83 gm, cod liver oil, 1 17 gm, thiamin ClHCl, 4 0 mg, riboflavin 0 4 mg, pyridoxine Cl, 0 3 mg, calcium pantothenate, 2 0 mg, cobalamine, 0 02 mg, and 2 me napthaquinone, 0 2 mg The diet is placed in a cloth bag in an inch thick layer and treated in the sterile lock by exposure to a 25 in vacuum for 10 min then free flowing steam for 5 min followed by 25 min steam sterilization at 17 p s i A vacuum is then drawn for 10 min and the food taken into the germfree cage or taken out for the conventional animals Food and water are presented *ad libitum* The animals given vitamin E supplements are fed about 30 mg α tocopherol per os semiweekly

In the first two experiments, Caesarean born, hand reared germfree rats were used, in the last two experiments, dam suckled germfree animals were used. None of the differences found between germfree and conventional rats seemed to disqualify *a priori* the germfree rat as a proper test animal

Results

The incidence of liver necrosis in the germfree and conventional rats is summarized in TABLE 1

* *Sponsored by the Commission on Liver Diseases, Armed Forces Epidemiological Board and supported in part by the Office of The Surgeon General Department of the Army and the Office of Naval Research*

TABLE 1
EFFECT OF NECROGENIC DIET ON GERMFREE AND CONVENTIONAL RATS

Status	Supplt to diet	Av init al wt in gm	Growth gm /day	Days on diet at death	No of rats dead with liver necrosis*/no started
rmfree	None	85	1 2	74 & 79	0/2
nventional	None	84 ± 5	0 7	165 ± 97	5/6†
rmfree	None	40	1 4	39 (34 & 43	0/2
nventional	None	42 ± 3	0 7	33 ± 3	8/8
rmfree	None	42	1 9	69	0/2
rmfree	Vit E	42	1 8	69	0/2
nventional	None	47 ± 4	0 5	41 ± 11	8/8
nventional	Vit E	54 ± 4	0 9	180	
rmfree	None	39	1 7	91 & 145	0/2
rmfree	Vit E	37	1 6	145	0/2
nventional	None	46 ± 5	0 8	68 + 23	12/12
nventional	Vit E	42 ± 4	1 0	150	0/4

* Incidence of necrosis based on histological examination by Doctor Harry Goldblatt
† One died with lung abscess

In the first experiment, rats with an initial weight of 85 gm were started on e necrogenic diet The germfree rats survived for some 74 and 79 days on et, and their livers were found to be normal The control rats however d not come down with necrosis until much later rendering the experiment conclusive The cause of death of the germfree animals was found to be as ciated with disturbances of the blood clotting mechanism as evidenced by

ntrol animals died with liver necrosis in an average time of 32 days The rmfree animals survived for 34 and 43 days One germfree animal had an ongated tooth and did not seem to eat well It was found moribund on the th day and the animal was killed Necropsy revealed hemorrhagic areas in e lungs The other animal was found to be moribund on the 43rd day The imal was bled from the abdominal aorta, and clotting time and prothrombin me were both found to be delayed compared to that of conventional rats The ers of both rats were found to be normal

Since vitamin E was the only vitamin lacking which was thought to be re ired during this period the third experiment included two germfree rats pplemented with vitamin E and two without supplement The eight con ntional controls promptly came down with necrosis in an average of 41 days he germfree rats ate more and gre some hat better than the conventional

TABLE 2
ALTERATIONS IN BLOOD CLOTTING AND HEMOLYSIS OF RED BLOOD CELLS IN GERMFREE AND CONVENTIONAL RATS

Status	Supplement	Days on diet	Clotting Time (Min.)		Hemolysis* after	
			Whole blood	Prothrombin*	10 hrs	20 hrs
Germfree	None	34	*			
	None	43	38	60		
	None	74	*	10		
	None	79	*			
	None	91	*		—	—
	None	145	80	60	—	—
	Vit E	145	12	5 9		
	Vit E	145	12	6 4		
Conventional	None	27			++†	++†
	None	68	0 5	0 5	+	+
	None	68	0 5	0 3		
	None	70	0 5	0 3		
	Vit E	113	0 5	0 3	—	—
	Vit E	113	0 5	0 3	—	—

* Clotting times were not taken in these animals however extensive hemorrhages found at death in lungs and other organs Their livers were normal
† 8 rats (all other data are expressed for individual rats)

In repeating the third experiment, 12 conventional control rats died with liver necrosis in an average time of 68 days No hemorrhages were evident in the two germfree rats supplemented with vitamin E The two unsupplemented germfree animals lived 91 and 145 days, respectively, and had no liver necrosis One animal died at 91 days after a blood sample was taken from the tail and on necropsy was found to have a stomach hemorrhage The other rat showed no pathology

The vitamin E stores of the unsupplemented germfree rats were estimated by the hemolysis test of Rose and Gyorgy [5] The negative hemolysis observed for these rats (TABLE 2) would indicate that the rats were not depleted of their stores of vitamin E

Discussion

Thus far we have been unable to induce liver necrosis in germfree rats using a diet which regularly produced lethal liver necrosis in conventional rats

The observed disturbances in the clotting mechanism of the germfree rats were suggestive of a deficiency of vitamin K In our experience, a vitamin K deficiency is not easily induced in germfree rats Although 40 per cent of the vitamin K may be destroyed by autoclaving as a dietary constituent,[3] the ... it is properly ...iency.[6] ... in E hemo... ...tes of con-

ventional rats fed the necrogenic diet normally hemolyze after 1–6 hours exposure to dialuric acid In simultaneous tests, erythrocytes of nonsupplemented germfree rats failed to hemolyze even after 20 hrs Such a negative hemolysis [illegible]

Summary

In a series of four experiments, eight germfree rats were found to have normal livers at autopsy All but one of the 34 conventional rats, fed the same diet, died with massive hemorrhagic necrosis of the liver The germfree rats exhibited prolonged clotting time which was partially prevented by vitamin E supplementation *

Acknowledgments

This work was supported, in part, by contract with the Office of Naval Research and the Army Medical Corps The authors acknowledge, with thanks, the help of Dr H A Gordon, Chief Pathologist, Morris Wagner, Chief Bacteriologist, B A Teah, Supervisor of Germfree Production and J Pleasants, biochemist from LOBUND Institute, and Dr Harry Goldblatt of the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles

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* Definite hemorrhagic necrosis of the liver was observed in five germfree rats since this report was given The germfree rats reported here ate almost twice as much as conventional rats whereas the germfree rats which developed liver necrosis were fed a diet restricted in quantity to that of the conventional control rats

HEPATIC INJURY DUE TO CONDITIONED SULFO AMINO ACID DEFICIENCY*

By Hans Popper, J de la Huerga, and Dieter Koch Weser

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Functional and structural changes resulting from nutritional deficiency of amino acids and, specifically, of sulfo amino acids are well known This paper will present examples of lesions resulting from drainage of sulfo amino acids from the body or from biological antagonism to them Since these functional and structural changes can be prevented or corrected by additional administration of relatively large amounts of the respective amino acids, they are considered examples of conditioned amino acid deficiency

Bromobenzene combined with cysteine is excreted in the urine after acetylation This represents a mechanism of depletion of cysteine and its precursors such as methionine from the liver and growth inhibition after administration of bromobenzene has been described[1,2] If 150 mgm of bromobenzene is given intraperitoneally to a fasting rat, an extensive centrolobular necrosis develops within 48 hours It is characterized by eosinophilic necrosis, hydropic swelling and, finally, disappearance of the liver cells associated with interstitial infiltration by inflammatory cells[3] (A in FIGURE 1) The lesion is more advanced in instances in which fasting for more than 12 hours precedes the bromobenzene injection and massive necrosis with hemorrhage imitating nutritional hepatic necrosis may be noted Administration of cysteine or methionine in large doses prevents the development of the lesion (B in FIGURE 1) This already suggests the possibility that drainage of sulfo amino acids rather than an intoxication with the halogenated benzene, causes the lesion Chemical [illegible] the histologic findings[4] In comparison to controls, the phos [illegible] ie esterase and succinic [illegible] intoxicated by bromobenzene [illegible] omsulfalein retention is noted In the animals treated simultaneously with methionine and cysteine most of these changes are either entirely prevented or at least reduced (FIGURE 2)

Additional evidence supports this concept The mercapturic acid excretion resulting from the administration of bromobenzene is markedly increased by the simultaneous administration of cysteine and methionine resulting in prevention of the hepatic lesion (TABLE 1) The greater portion of bromobenzene is detoxified by means other than mercapturic acid excretion such as formation of glucuronates or sulfates Nevertheless only a small increase of the fraction of bromobenzene detoxified as mercapturic acid is associated with the protection This suggests that the protection is not due to the removal of relatively small amounts of bromobenzene by detoxification via mercapturic acid but rather is due to repletion or sparing of the sulfo amino acids in the hepatic pool In the case of glycine used for hippuric acid formation, such a pool of 10

* Supported by a grant from the Doctor Jerome D Solomon Memorial Research Foundation



FIGURE 1 Rat liver A ×120 48 hours after administration of bromobenzene F[illegible] The [illegible] hydropic swelling of the liver cells and inflammatory [illegible] is seen B ×120 48 hours after administration of bromobenzene and methionine The architecture is not disturbed

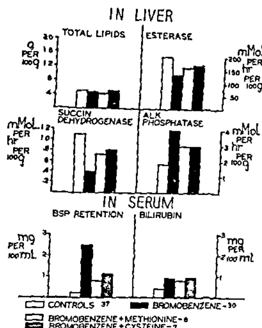


FIGURE 2. Results of chemical determinations on liver tissue and serum of control rats and rats sacrificed 48 hours after administration of 150 mgm. bromobenzene/100 gm. body weight with or without supplemental administration of 300 mgm. methionine or cysteine/100 gm. body weight. (Small figures in the legend indicate the number of animals.)

TABLE 1

URINARY MERCAPTURIC ACID EXCRETION OF CONTROL RATS AND OF ANIMALS WHICH HAD RECEIVED 48 HOURS BEFORE 150 MGM. BROMOBENZENE PER 100 GM. BODY WEIGHT WITH OR WITHOUT ADDITIONAL ADMINISTRATION OF 300 GM. METHIONINE OR 300 GM. CYSTEINE/100 GM. BODY WEIGHT

	Mercapturic acid	
	As mgm. cysteine	As % bromobenzene injected
Controls	1	—
Bromobenzene	32	13.9
Bromobenzene + Methionine	53	23.2
Bromobenzene + Cysteine	52	23.1

mg./100 gm. body weight has been demonstrated.[5] In the case of bromobenzene, 30 mgm. cysteine are made available for mercapturic acid formation.

The cysteine binding takes place on the para position of the bromobenzene molecule. Consequently, benzenes dihalogenated in the para position such as para dibromobenzene and para chlorobromobenzene do not produce the lesion. In contrast, meta dichlorobromobenzene with the para position free produces it (TABLE 2). This is confirmed by low mercapturic acid production with the para compounds and high production with the meta compound. Again, the

TABLE 2

URINARY MERCAPTURIC ACID EXCRETION OF RATS DURING 48 HOURS FOLLOWING THE ADMINISTRATION OF EQUIVALENT AMOUNTS OF BROMOBENZENE OR DIHALOGENATED BENZENES

	Mgm /100 gm body weight	Mercapturic acid	
		Amount mg	As % of compound administered
Bromobenzene	75	18	15 6
m chlorobromobenzene	91	1	16 5
p-chlorobromobenzene	91	5	4 3
p dibromobenzene	112	4	3 5

percentage of the halogenated compound excreted as mercapturic acid is rather small These findings militate strongly against a primary toxicity of the halogenated benzenes but support drainage of sulfo amino acids as the cause for the hepatic necrosis Finally in animals in which spontaneous liver damage occurred as reflected in focal necroses apparently due to infection bromobenzene injection failed to produce the characteristic necrosis If the concept presented is correct the process of detoxification as such may become detrimental to the liver

Another example of conditioned amino acid deficiency in these sense described above is the effect of ethionine an amino acid similar to methionine except that the terminal methyl group is replaced by an ethyl group This supposed biological antagonist to methionine[6] has been shown to produce pancreatic necrosis followed by pancreatic fibrosis and atrophy[9] and dilatation of the pancreatic ducts upon prolonged treatment In late stages the acinar cells reveal an irregular regeneration Whereas in early stages the islets appear entirely normal hydropic swelling of the island cells results in some of the animals treated for longer than one month (FIGURE 3) In some of these animals the urine contains reducing substances However the blood sugar curve is normal and further investigations are required to elucidate this glycosuria These lesions in an organ as sensitive to protein deficiency as the pancreas are prevented or corrected by methionine administration

Intraperitoneal administration of single doses of ethionine produces fatty liver in female rats but not in males Females are protected by administration of testosterone and males are made sensitive by castration This points to a protective effect of male sex hormone as far as the acute fatty liver is concerned It can best be explained by a protein sparing effect of testosterone preventing the f



FIGURE 3 (×150) Pancreas of rat wh ch had been on 0 5 per cent eth on ne diet for 51 davs There is hy dropic swelling of the island cells distort on of the acini w th marked regenerat on of the acinar epithel um d a tat on of the ducts and interst t al inflammatory nfiltration

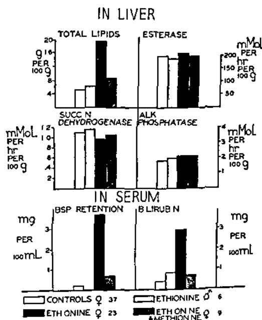


FIGURE 4 Results of chemical determinations on l ver tissue and serum in control rats as well as in rats wh ch had received 100 mgm eth onine per 100 gm body weight 48 hours before sacr ficing with or without sup plemental admin tration of 300 mgm meth on ne per 100 gm body we ght (Small figures in the legend ind cate the number of an mals)

liver develops within approximately one week. After about 12 days, fat is seen only in the central lobular zone. However, now there is central necrosis and streamers of interstitial infiltration extending into the lobular parenchyma chiefly around intralobular cholangioles (A in FIGURE 5). After three weeks extensive interstitial infiltration around the cholangioles is associated with marked liver damage and, after four weeks on the diet, a diffuse hepatic lesion is noted in male as well as in female rats.[15] It is characterized by diffuse hepatocellular damage with hemogenization of the cytoplasm recognized by reddish discoloration in Mallory's aniline blue or Gomori's trichrome stained section (B in FIGURE 5). This damage often progresses to cytolysis and is also associated with bizarre regeneration of the liver cells which reveal large nuclei and large and often multiple nucleoli (A in FIGURE 6). Moreover there is a dense interstitial infiltration by reticuloendothelial cells. In the center of the lobules lipofuscin deposition reflects preceding central necrosis. The fibers of the reticulum framework reveal extensive proliferation. However dissection of the lobules by septa is only occasionally seen. There is increased visibility of intralobular cholangioles, the presence of which in the normal liver was recently emphasized by Elias.[16] These intralobular cholangioles are not easily seen but become readily apparent if the common duct is ligated. The septal and interlobular bile ducts proliferate and the latter may reveal marked dilatation (B in FIGURE 6). This is also associated with a marked dilatation of the large extrahepatic biliary duct to about 2 mm in diameter, similar to the changes recently described in mice bearing grafted pituitary tumors.[1]

The characteristic lesion described is associated with reduced fat and protein content, low esterase and high phosphatase activity in hepatic tissue and increased bilirubin, markedly high alkaline phosphatase and low esterase activity in the serum. The morphologic and chemical alterations can be prevented if one per cent methionine is added to the diet or when the animals are returned for one week or longer to the stock diet (FIGURE 7). During the course of the chronic ethionine intoxication the hepatic lipids first show a peak and subsequently a depression below the normal level whereas the proteins consistently decrease (FIGURE 8).

The characteristic diffuse hepatocellular degeneration with cholangiolitis and interstitial infiltration is subject to various influences. Only few rats survive the simultaneous administration of cortisone and ethionine, since most of them die during this period from focal hepatic necrosis apparently due to infection and not due to ethionine. However rats which survive the simultaneous administration have nearly normal livers. Administration of large doses of choline with the diet prevents the inflammatory changes but produces marked regenerative changes of the liver cells. The hepatic lesion caused by chronic ethionine feeding is not significantly altered by administration of pancreatin, vitamin B, penicillin, or a high fat or low protein diet. It is markedly ag





FIGURE 6 Rat livers A ×235 After 35 days on the 0.5 per cent ethionine diet The liver cells show cytolysis as well as irregular regeneration The nucleoli are large The cholangioles are proliferated There is extensive interstitial infiltration by round cells B ×66 After 1 days on the 0.5 per cent ethionine diet Marked dilatation of larger bile ducts is noted

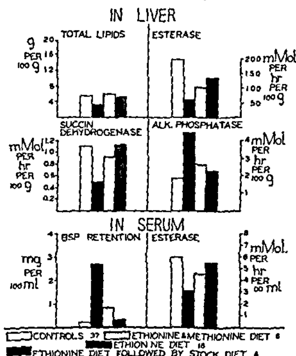


FIGURE 7. Result of chemical determination on liver tissue and serum in control rats as well as in rats which have been on the 0.5 per cent ethionine diet for 28 days with or without supplemental 1 per cent methionine and in rats which have been on the ethionine diet for the same period and subsequently on the stock diet for 10 days. (Small figures in the legend indicate the number of animals.)

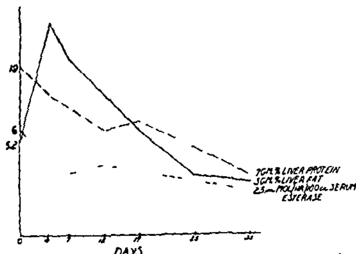
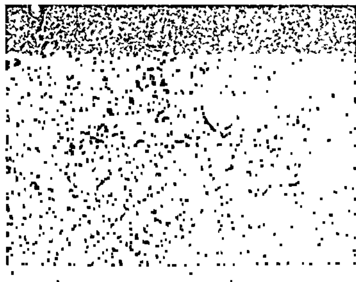


FIGURE 8. Protein and fat in hepatic tissue and esterase in serum in rats for various periods on 0.5% ethionine diet.

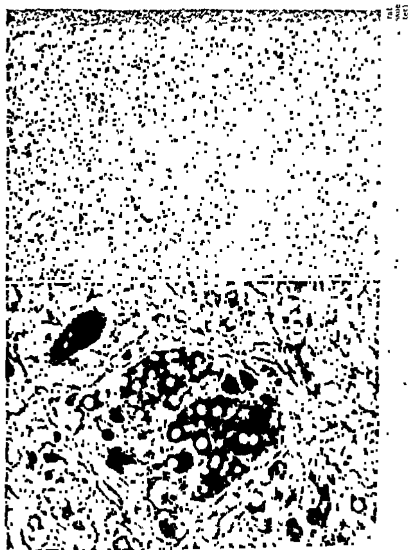


been kept for approximately 100 days on diets containing 0.2 per cent ethionine, the lesions are markedly intensified The bile duct epithelium may reveal bizarre and excessive proliferation characterized by irregular mitoses and by

contrast to the extensive fiber proliferation between single cell plates in the surrounding liver tissue (A in FIGURE 10) These groups of cells may become

proliferations, no information is as yet available as to the biological behavior of these liver cell nodules Nevertheless their histologic characteristics strongly suggest them to be hepatomas at least of the benign variety Further study is required of these bile duct and liver cell nodules Usually, more than one such lesion has been found in the 17 rats with nodules Their response to methionine treatment particularly requires study

Thus, the ethionine lesion imitates the picture of human malnutrition, especially kwashiorkor, since it starts with fatty metamorphosis, proceeds



…rotic changes, and terminates in lesions at least suspicious for hepatic tumor …mation, and since it is associated with pancreatic alterations An amino acid …agonism has to be assumed rather than a toxic effect of ethionine in produc… these lesions, although the latter cannot be completely excluded Triethyl…ne, a possible metabolite of ethionine 11 does not produce hepatic and pan…c alterations under similar conditions …us appears that most of the available evidence militates against a toxic …f both bromobenzene and ethionine and suggests that depletion of, or …ism to, sulfo amino acids is the basis of the lesions demonstrated in …xicity and deficiency meet The lesions represent patterns of a con… amino acid deficiency in a broad sense of "malnutrition caused by … other than a primary dietary deficiency" 18 It may be worth while …for a similar pattern in man which might explain lesions which are …nutritional deficiency but to internal interference with amino acid metabolism

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CLINICAL EVALUATION OF A HIGH PROTEIN HIGH CARBOHYDRATE RESTRICTED FAT DIET IN THE TREATMENT OF VIRAL HEPATITIS*†

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Introduction

The relationship between diet and certain types of liver injury has been established in the case of animals [1 2 3 4 5] and specific liver protective factors have been described [4 6 7] Results of such studies however, do not appear to be directly applicable to viral hepatitis in man because this disease has not been transmitted successfully to animals The clinical importance of diet in the treatment of hepatitis in man has often been emphasized but the degree of this has not been entirely clear The quantity of fat in the diet has been

what amounts to a clinical trial involving a number of variables [a] the pro longed nature of such studies in hepatitis the lack of early specific diagnoses in many situations [15] and the absence of established uniform methods of evalua tion

The present study was directed towards the evaluation of the effect of a high

Epidemiological Board and with the cooperation of the Bureau of Priso U S Department of Justice The findings reported here are the product of study of 67 controlled cases of induced homologous serum hepatitis which oc curred among volunteers in two institutions

Conduct of Study

The volunteer participants were men between the ages of 21 and 35 In the latter part of the study men of 30 years and over were not accepted Final selection was based upon a personal interview a medical history a physical examination and a review of the available records to determine the suitability

of the volunteers on the basis of age, physical condition mental status and personality In addition complete series of liver function tests were run in cluding serum bilirubin (one minute and total), thymol turbidity, cephalin flocculation, thymol flocculation, total lipids, zinc turbidity, and urine urobilinogen and bilirubin

The nature of the over all project made it possible to observe the course of the disease from the earliest appearance of abnormal laboratory or clinical findings It was possible, therefore, to study the effects of diet starting at a much earlier period in the illness than could normally be possible in the case of casual hospital patients Every effort was made to take advantage of this feature of the study by placing patients on a dietary regimen as early as possible in the course of their illness Good control was achieved and the design was such as to provide for the collection of data in terms of well defined periods in the patient s course from pre inoculation to discharge

Throughout the entire study, two diets applicable or adaptable for clinical use were employed As the subjects manifested signs or symptoms of hepatitis, they were admitted to the hospital and placed on a high protein high carbo hydrate, restricted fat diet, referred to here as the special diet or on an ad lib" diet

mum voluntary food consumption.

The 'ad lib' diet consisted of the same food provided for the general institu tion population There were no restrictions made on the type character quantity or quality of food other than the general limitations of the institution diet

Subjects remained on the respective diets from the time of admission until

TABLE 1

	Special diet				Ad lib diet				Difference			
Period	Cal	Protein	Fat	CHO	Cal	Protein	Fat	CHO	Cal	Protein	Fat	CHO
Acute	3616	203 3	78 8	541 5	2213	77 5	78 2	30 3	1403 0	125 8	0 6	234 2
Recovery	3954	221 0	80 1	599 0	2833	102 6	98 0	392 0	1121 0	118 4	18 1	206 9
Total	3844	215 2	9 2	5 9 4	2609	93 8	90 5	361 7	1235 0	121 4	14 5	217 7

TABLE 2
DIETARY VALUES IN GRAMS

Sampling period (days)*	Diet	By calculation		By chemical analysis	
		Protein	Fat	Protein	Fat
7	Special	210 5	86 3	192 3	68 6
7	Ad lib	111 3	110 7	94 9	96 9
7	Special	209 3	87 8	198 1	76 7
7	Ad lib	101 6	100 9	92 9	77 6
7	Special	215 0	97 0	193 0	100 7
7	Ad lib	106 5	106 9	100 5	76 0
7	Special	203 7	93 9	212 1	80 2
7	Ad lib	100 2	110 1	92 8	76 3
7	Special	254 9	116 1	219 2	117 0
7	Ad lib	100 8	107 1	94 3	81 5
7	Special	241 4	122 1	209 6	97 7
7	Ad lib	105 4	119 3	97 5	87 0
7	Special	221 3	84 3	221 8	90 6
7	Ad lib	113 3	106 0	91 6	95 9
7	Special	208 0	93 1	190 9	67 9
7	Ad lib	127 6	138 8	96 2	96 8
5	Special	171 8	68 3	229 0	91 1
5	Ad lib	104 3	101 3	96 7	75 2
5	Special	190 5	70 9	226 6	85 3
5	Ad lib	118 3	96 9	133 1	73 9
1	Special	253 3	61 0	288 6	105 2
1	Ad lib	116 8	104 8	104 6	78 0
2	Special	229 8	110 2	223 2	103 7
2	Ad lib.	99 2	95 9	86 3	81.3
2	Special	221 3	72 0	311 1	61 7
2	Ad lib	133 9	141 2	139 8	103 0

* Complete meals were homogenized in a blender Aliquots of each meal were pooled for the period indicated and samples of these pools set aside for chemical analysis

under supervision Edible waste or uneaten food was also weighed and the amount recorded Food intake figures were calculated for each patient using these data Sample meals representative of both diets were chemically analyzed for comparison with the calculated values *

Sixty seven volunteers who developed hepatitis with jaundice were included in the present study Of these, 32 were in the "special" diet group and 35 in the "ad lib " diet group During the time this study was being conducted, a total of 85 individuals was started on one or the other of the dietary regimens

A number of these patients were already in the hospital with hepatitis when the dietary study was commenced Since only those cases in which it was possible to calculate dietary intakes for the complete period of hospitalization were considered for inclusion in the group, eleven of these initial subjects were excluded, seven on the "special" diet and four on the "ad lib" diet Two further cases, both on the "special" diet, were excluded because of uncertainty as to their dietary intake,* as were five individuals for whom a diagnosis of hepatitis with jaundice could not be sustained All of these 18 individuals were eliminated before the statistical analysis was made Initially, the distribution of patients between the two diet groups was on a one-for one basis, a separate series of alternations being followed in each of the two institutions Due to the imblance of the numbers between the two diet groups occurring as a result of the elimination of the above 18 individuals (five on the "ad lib" diet and 13 on the "special" diet), the last ten patients admitted to the dietary study were distributed on a two-for-one basis

The cases of heptitis studied resulted from parenteral inoculation of the following infecting materials

A *Infected pool plasma* A single lot of infected plasma was used in all the work relating to the safety of blood products This pool was prepared from commercially procured plasma to which had been added some 800 ml of plasma containing the "Fort Bragg" strain of homologous serum hepatitis virus as well as a total of approximately 600 ml of plasma or serum from four reputed cases of serum hepatitis The original volume of the material was approximately 130 liters, and it is referred to here as infected pool plasma" The subjects who received this material represent a homogeneous group

B *Infected pool plasma, treated* This represents materials which remained infective after treatment by one of the following processes

(1) Ultraviolet irradiation at various levels

(2) Heating for 2 hours at 60° C,

(3) Heating for 4 hours at 60° C

(4) Dilution to 10^{-3} and 10^{-4}

(5) Treatment of β propiolactone at different levels or

(6) Storage at room temperature for 3–6 months

Subjects who received these materials represent a heterogeneous group in the sense that, although they all received infected pool plasma this had been treated in a variety of ways each presumably affecting the virus in different degree

C *Carrier material* Six suspected carriers of homologous serum hepatitis were examined by inoculation of their plasma into volunteer subjects Five of the six were proved to be carriers In addition, a few cases resulting from first passage infected pool material have been placed in this group

Cases resulting from the inoculation of some of the carrier materials" had short incubation periods in the range 30 to 70 days, whereas those who received infected pool plasma had incubation periods between 60 and 120 days. This difference in incubation periods coupled with a somewhat different cl

* One of these subjects received infected pool plasma the other infected pool plasma treated Th during which their total serum b l rub n levels remained above 1 0 mgm per 100 ml were 63 days respectively

picture in which the severity of symptoms at the onset of illness was greater in relation to the laboratory findings than would be expected on the basis of our experience with the other infecting materials studied, suggests that *some of the* cases receiving "carrier material" may have had infectious hepatitis induced by parenteral inoculation

D *Thrombin* Thrombin from a commercial batch reported to have caused cases of hepatitis in clinical use was inoculated into ten subjects in order to determine the safety of thrombin with respect to homologous serum hepatitis Four of the subjects who developed hepatitis were studied from the dietary point of view They do not fall into *any of the above groups, so it is* difficult to evaluate them except from the over all point of view as to the development *of complications*

The distribution of the 67 cases between the two diet groups, broken down by [illegible] at the foot of TABLE 3A

for the study

(2) The incubation period, beginning with inoculation and extending to the time at which a diagnosis of hepatitis could be made

(3) The period of illness which was divided into the acute period and the recovery period

These periods were defined roughly by changes in liver function tests and, therefore, permitted objective comparisons

Cases of all degrees of severity were encountered including three which ended fatally These latter cases had been respectively inoculated with infected pool plasma, infected pool plasma, treated, and carrier material

TABLE 3A
DURATION OF ILLNESS IN DAYS—TSB 1 0 TO 1 0

Infected pool plasma		Infected pool plasma treated		Carrier material		*Thrombin*	
Spec	Ad lib	Spec	Ad lib	Spec	Ad lib	Spec	Ad lib
79	78	36	70	63	46	22	25
62	86	49	56	81	49		13
85	86	63	130	92	35		27
98	102	42	84	21	28		
93	70	56	49	30	43		
*	47	28	56	74	24		
102	45	53	47	*	47		
105	45	15	46	56			
94	63	45	18				
	50	*	70				
		15	67				
		35	21				
		14	42				
		98	22				
			59				
Total No Cases 9	10	14	15	8	7	1	3

* Fatal cases (not included in the analysis)

The statistical evaluation of the effect of the two diets was made in terms of the duration of illness of the 67 cases It should be noted that in such a comparison when recovery is the end point it is not feasible to include cases which do not recover For this reason the three fatal cases are excluded from this analysis although it is obvious that they must be taken into account when evaluating the results

Discussion of Statistical Methods

Most of the analysis was based on measures of duration such as the number of days during which liver function tests particularly the total serum bilirubin (TSB), remained abnormal or the number of days of clinical illness *etc* The data have been examined in a number of ways to try and discern differences between the cases on the "special" diet and those on the ad lib diet and

TABLE 3B

DURATION OF ILLNESS—TSB 1 0 TO 1 0

Diet	Infected pool plasma	Infected pool plasma treated	Carrier	Thrombo	Sum
Special x	718	549	417	22	1706
$\bar{x}_1$	89 7500	42 2308	59 5714	22 0000	58 8276
n_1	8	13	7	1	29
Ad lib x_2	672	837	272	65	1846
$\bar{x}_2$	67 2000	55 8000	38 8571	21 6667	52 7429
n_2	10	15	7	3	35
Sum X	1390	1386	689	87	3552
$\bar{x}$	77 2222	49 5000	49 2143	21 7500	55 5000
N	18	28	14	4	64
$d = \bar{x}_1 - \bar{x}_2 =$	22 5500	−13 5692	20 7143	0 3333	
$w_i = \frac{n_1 n_2}{N} =$	4 4444	6 9643	3 5000	0 7500	15 6587
$w d_i =$	100 2212	−94 5000	72 5946	0 2500	78 5658
$w d^2 =$	2259 9881	1281 2894	1505 7064	0 0833	5048 0672

$(\Sigma w d)^2/\Sigma w = 394\ 1952$

ANALYSIS OF VARIANCE

Source	Sum of squares	df	Mean square
Diet ignoring material	587	1	
Interaction	4654	3	1551 29
Material	14414	3	4804 67
Between cells	19655		
Within cells	27455	56	490 2
Total	47110	63	
Mean	197136	1	

Mean square	df	Sum of squares	Source
	3	14607	Material ignoring diet
1551 29	3	4654	Interaction
394	1	394	Diet

Interaction $F = \frac{1551\ 29}{490\ 27} = 3\ 16$ $F_{05} = 2\ 8$

TABLE 3c
DURATION OF ILLNESS—TSB 1 0 TO 1 0

Source	SS	df	MS	
			Infected Pool Plasma	
Mean	107339	1		
Total	7397	17		
Diet	2260	1	2260	$F = \frac{2260}{321} = 7\ 04$ $F_{05} = 4\ 49$
Within diet	5136	16	321	
			Infected pool plasma, treated	
Mean	68607	1		
Total	18789	27		
Diet	1282	1	1282	$F = \frac{1282}{673} = 1\ 90$ $F_{05} = 4\ 22$
Within diet	17507	26	673	
			Carrier	
Mean	33909	1		
Total	6189	13		
Diet	1501	1	1501	$F = \frac{1501}{391} = 3\ 84$ $F_{05} = 4\ 75$
Within diet	4697	12	391	

TABLE 3D
DURATION OF ILLNESS—TSB 1 0 TO 1 0

Source	SS	df	MS	
			Special Diet	
lean	100360	1		
otal	24558	28		
laternal	12590	3	4197	$F = 8\ 76$ $F_{05} = 2\ 99$
esidual	11968	25	479	
			Ad lib diet	
lean	97363	1		
otal	21965	34		
laternal	6477	3	2159	$F = 4.32$ $F_{05} = 2\ 91$
esidual	15488	31	500	

hen differences existed, to determine their statistical significance A "five er cent" level of significance was decided upon before any calculations were ndertaken This implies that a difference, measured in terms of its standard ror, could have occurred by chance only once in 20 times.

Tables 3A, 3B, 3C, and 3D illustrate the analysis in the case of the duration of ness as judged by the time the total serum bilirubin remained above 1 0 mgm r 100 ml

Three fundamentally separate tests have been employed in this analysis e tests for duration, body weight change, and the incubation period

It was apparent early in the analysis that the variation in the measures selected for study was influenced by the type of infecting material as well as by diet The analysis of the data relating to the period before diets were started brings out a number of differences associated with the type of infecting material One such characteristic is the incubation period The average incubation periods were infected pool plasma, 81 days, infected pool plasma, treated, 103 days, and carrier material, 61 days

Another characteristic of the differences between infecting materials which is free of diet effect is body weight loss during the incubation period The respective average differences are infected pool plasma, −5 4 lbs , infected pool plasma, treated, −5 5 lbs , and carrier material, −3 6 lbs

The weight loss data are based on fewer than the 67 patients, because either the inoculation or hospitalization weight was not obtained in a few instances However, if the assumption is accepted that a missed weight could occur with the same probability in the group receiving different infecting materials, then the available numbers are sufficient to establish the difference between infecting materials

The data on incubation periods and on loss of body weight occurring during the incubation period can be used to test whether bias was introduced in the assignment of patients to the diets If bias existed, it might result in a preponderance of patients with short incubation periods being assigned to one of the diet groups No such difference was found Similarly, there was no difference between diets as measured by weight loss during the incubation period

Results

A *Food consumption levels* TABLE 1 summarizes the calculated food intake levels in terms of average consumption during the acute period, the recovery period, and for the total period of illness It will be seen that differences of well over 1,000 calories, 100 gm in the case of protein and over 200 gm in the case of carbohydrate, were maintained between the two diets

B *Chemical analysis of diets* Chemical analyses of the diets were made to confirm calculated values These are compared in TABLE 2

C *Effect of diet* In the course of the analysis, significant interaction between diet and the different infecting materials was found that is, the effect of diet was not consistent with respect to infecting material Where this interaction appeared in the analysis, it was necessary to analyze separately the effect of diet within each group which received different infecting material The following measures of effect were used

(a) The number of days the TSB remained abnormal when 1 0 was taken as the endpoint (duration of time the TSB was elevated above 1 0 mgm per 100 ml)

(b) The number of days during which the bilirubin remained abnormal when 1 5 mgm per 100 ml was taken as the endpoint As a corollary to this, the period of time taken for the TSB to fall from 1 5 to 1 0 was studied

(c) The number of days for the TSB to reach its maximum level

(d) The number of days for the TSB to return form maximum to 1 0 mgm per 100 ml.

(e) The number of days during which the patient was ill, *i.e.*, from the time of onset of illness until the patient could be said to be improving clinically

(f) The duration of the recovery period, *i.e.*, from time of the onset of clinical recovery until the bilirubin reached 1.0

(g) Weight change during illness

(h) Average maximum TSB levels in each of the diet groups. This measure of effect can also be used in part as a test for bias

The above measures of effect have been analyzed statistically. It must be emphasized that (a) through (g) are interrelated and do not constitute separate analyses. They represent measures of the duration of illness or portions of it as judged by different end points

The results are summarized in TABLE 4. They reveal the following

(1) With return to a bilirubin level of 1.0 mgm. per 100 ml. taken as the

TABLE 4
EFFECT OF DIET

Measure of effect	Infecting material	Average: Special diet	Average: Ad lib diet	Remarks
		Days†		
Duration of illness (TSB 1.0 to 1.0)	IPP*	90	67	*Significant* diet effect
	IPP, treated	42	56	No significant diet effect
	'Carrier'	60	39	No significant diet effect
Duration of illness (TSB 1.0 to 1.5)	IPP	72	52	No significant diet effect
	IPP, treated	24	36	No significant diet effect
	'Carrier"	45	23	*Significant* diet effect
Duration from TSB 1.5 to 1.0	All groups	17	16	No significant diet effect
Duration from TSB 1.0 to max.	All groups	20	16	No significant diet effect
Duration from max TSB to 1.0	All groups	39	37	No significant diet effect
Acute period	All groups	14	17	No significant diet effect
Recovery period	IPP	55	47	No significant diet effect
	IPP treated	25	35	No significant diet effect
	'Carrier'	41	21	No significant diet effect
Incubation period	All groups	85	88	No significant difference between diet groups
		mgm./100 ml		
Maximum TSB	All groups	11.0	9.7	No significant difference between diet groups
		lbs		
Wt. change between inoculation & TSB 1.5	IPP	1.4	−8.6	*Significant* diet effect
	IPP, treated	−2.1	−6.3	*Significant* diet effect
	'Carrier'	−1.0	1.1	No significant diet effect

* Infected pool plasma † To nearest whole day

endpoint of recovery

(a) Subjects who received infected pool plasma and were given the "special" diet had a course of illness which was on the average 23 days longer than the corresponding individuals on the "ad lib" diet This difference was statistically significant

(b) Subjects who received infected pool plasma, treated, and were given the "special" diet had a course of illness which was on the average 14 days shorter than corresponding individuals on the "ad lib" diet This difference did not reach statistical significance

(c) Subjects who received "carrier material" and were given the "special" diet had a course of illness which was on the average 21 days longer than corresponding individuals on the "ad lib" diet This difference was not statistically significant.

(2) With return to a bilirubin level of 1 5 mgm per 100 ml taken as the point of recovery, results were similar and in the same direction in each of the categories listed in 1(a) (b) and (c) above The differences however reached statistical significance only in the case of "carrier material" When the period between the 1 5 TSB and 1 0 TSB levels was studied, no statistically significant effect of diet could be demonstrated.

(3) In calculating the average duration of time between a bilirubin level of 1 0 and the maximum TSB no statistically significant diet effect was demonstrable

(4) In calculating the average duration of time between the maximum TSB and the endpoint of recovery at 1 0 no significant diet effect was demonstrable

(5) In calculating the average duration of the acute period no statistically significant diet effects were apparent Because of interaction however it was necessary to make separate calculations with respect to infecting materials when examining the average recovery periods Here the differences were analogous to those obtained in 1(a), (b) and (c) However the differences were not statistically significant

(6) There were no significant diet effects on weight change during the period from hospitalization to the fall of TSB to 1 5

D *Effect of infecting materials* The statistical evaluation disclosed a marked effect due to the type of infecting material used Whenever interaction was present it was necessary to carry out the analysis with respect to each of the measures of effect within each diet group separately These results are summarized in TABLE 5 Reference to this table discloses the following

(1) Within each diet group, there was a significant effect due to the infecting material used whether the endpoint of the duration of illness was measured by the return of the TSB level to 1 5 or to 1 0

(2) There was a significant effect of the infecting material on the period between a TSB level of 1 5 and a TSB level of 1 0

(3) There was no significant effect of infecting material on the average number of days from 1 0 to the maximum TSB level There was however a significant effect on the average number of days from the maximum TSB level to

TABLE 5

EFFECT OF INFECTING MATERIAL

Measure of effect	Diet	Average: Infected pool plasma	Infected pool plasma treated	Carrier	Remarks
		Days*			
Duration of illness (TSB 1 0 to 1 0)	'Special' "Ad lib"	90 67	42 56	60 39	*Significant* effect *Significant* effect
Duration of illness (TSB 1 0 to 1 5)	'Special' "Ad lib"	72 52	24 36	45 23	*Significant* effect *Significant* effect
Duration from TSB 1 5 to 1 0	Both	16	19	15	*Significant* effect
Duration from TSB 1 0 to max	Both	24	15	17	No significant effect
Duration from max TSB to 1 0	Both	53	34	33	*Significant* effect
Acute period	Both	17 5	12	18	No significant effect
Recovery period	'Special' "Ad lib"	55 5 47	25 35	41 21	*Significant* effect *Significant* effect
Incubation period	Both	81	103	61	*Significant* effect
		mgm /100 ml			
Maximum TSB	Both	15 4	8 0	10 1	*Significant* effect
		lbs			
Wt change between Inoc & TSB 1 5	"Special" "Ad lib"	1 4 −8 6	−2 1 −6 3	−1 0 1 1	No significant effect *Significant* effect

* To nearest whole day

(3) The same effects were apparent when the period of illness was broken down into acute and recovery phases

(4) There was a significant effect of infecting material on the average maximum TSB levels

(5) There were significant differences between the mean incubation periods of the infecting materials used

(6) There was no significant effect on weight changes of infecting material during the period between hospitalization and fall of TSB to 1 5

E *Relation between diet and the development of complications* All cases on the dietary study were evaluated with respect to development of the following complications death, coma, pre coma, recrudescence, relapse, prolonged illness (more than 4 months), and residua persisting beyond the time of discharge from the program The latter included persistent hepatomegaly, splenomegaly, right upper quadrant pain or tenderness, or failure of hepatic tests to resume pre inoculation levels Since these complications cannot be given equal

TABLE 6

Definitions	Special no	Diet %*	Ad lib no	Diet %*
[illegible]	4	12 5	0	0
[illegible]	5	15 6	1	2 9
[illegible]	13	40 6	5	14 3
[illegible]	15	46 9	6	17 1
[illegible]	15	46 9	7	20 0
[illegible]	18	56 2	8	22 8

All but one of the above definitions (*B*) yield statistically significant differences indicating a higher occurrence of complications among those who received the special diet

* In terms of the total number cases in each diet group

weight, a number of definitions employing increasingly restrictive criteria were applied The definitions and the number of cases falling into each are shown in TABLE 6

The definitions listed are to some extent arbitrary and as they are not independent it must not be assumed that the data have been subjected to six

diet

In compiling this table cases were counted only once Cases with more than one of the listed complications were counted only under the most restricted definition Two of the cases listed under definition A require special mention These subjects were members of a group of five volunteers who had been inoculated with plasma obtained from a proved carrier who had developed hepatitis with jaundice some 6 months before the plasma was collected These two cases developed fulminating hepatitis with incubation periods of 44 and 46 days One of the cases terminated fatally The remaining three subjects in the group did not develop hepatitis The unusual severity of the illness produced by this particular inoculum suggests, though this cannot be proved that the outcome was related more to the infecting material than to the diet Even if the two cases are removed from the table however, the over-all results are not appreciably changed.

Discussion

All of the cases of hepatitis included in the present study were of so-called "inoculation hepatitis" Most were undoubtedly serum hepatitis, but there is some reason for believing that the illness resulting from the inoculation of some of the "carrier materials" may have been infectious hepatitis Because of this the broader term "viral hepatitis" has been used in the title

The present cases developed in the course of a study on the sterilization of blood and blood products This study was limited in scope and there was no way of knowing beforehand how many cases of hepatitis could be anticipated Consequently no effort was made to randomize patients with respect to both

diet and infecting material. A simple one-for-one allocation of patients between the diet groups was followed, in the hope that a practical randomization would thereby occur. A study of the incubation periods of patients who received different inoculating materials (a measure free of the effect of diet) indicates that this objective was indeed achieved.

The special feature of the present study is the fact that it was possible to place patients on one or other of the dietary regimens at the earliest appearance of signs, symptoms, or laboratory findings suggestive of hepatitis. This institution of dietary therapy must be taken into consideration in any interpretation of the results of the present study.

The severity and duration of illness in viral hepatitis are extremely variable. In the present study the analysis was based only on objective findings such as the duration of elevation of the total serum bilirubin, and it should again be emphasized that although different endpoints and subdivisions of this measure of effect were used in the analysis, these are not independent but are merely parts of the same analysis.

Summary

(1) A group of 67 volunteers taking part in studies on viral hepatitis has been evaluated with respect to two diets: a high protein, high-carbohydrate, restricted fat diet and an "ad lib." diet.

(2) It was possible to institute the diet in each case at an early point in the illness.

(3) The type of infecting material had a significant effect on most of the measures of duration studied.

(4) Apparent differences in effect of the two diets did not reach the level of significance with most of the measures of duration studied. In those instances where this did occur, the average duration of illness of patients on the "ad lib." diet was shorter.

(5) The occurrence of complications was more frequent among patients receiving the high protein, high-carbohydrate, restricted fat diet.

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